Dynamic Interactions Between Stem Cells and Biomaterials

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Abstract

The cellular microenvironment has been known to direct the cell behaviour through biochemical and mechanical signalling. Different biomaterials have been fabricated to study the impact of biophysical cues on proliferation and stem cell differentiation in vitro. Stem cells have immense promise in regenerative medicine. Therefore, there is a pressing need to understand the interdependency of biophysical signals and biochemical signals in regulating stem cell potency and differentiation. In this chapter, we explore the different types of biomaterials commonly used for studying mechanobiology in stem cells and highlight the primary mechanism and pathways behind extracellular matrix (ECM)-mediated cellular response. Furthermore, we discuss how the understanding of stem cell mechanobiology influences the fields of tissue engineering and regenerative medicine. We also touch upon the importance of mechanobiology in cancer. In short, we have tried to convey to our readers that although current expansion and differentiation methods use biochemical molecules alone, it is crucial to understand that biophysical cues from the stem cell microenvironment can also regulate the proliferation and differentiation of stem cells.

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15.1 Introduction

Human pluripotent stem cells (hPSCs), which include both human-induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs), have a unique ability to differentiate into cells of three germ layers and have unlimited expansion potential; hence, they can be used for tissue engineering. Multipotent stem cells, for example, mesenchymal stem cells and hematopoietic stem cells, are often used for various clinical researches, and there are several clinical trials conducted with these cells. However, most applications remain at the clinical trial stage due to the non-functionality of transplanted cells, cell death after transplantation, deposition of cells into the lungs, or teratoma formation (Lodi et al. 2011; Naji et al. 2019). This can be due to sudden changes in the microenvironment from in vitro to in vivo. Many researchers have been trying to study interactions between stem cells and their surrounding microenvironment to overcome this.

In vivo, stem cells reside in a specific microenvironment, also known as "niche." This niche maintains an equilibrium between stem cell self-renewal and differentiation and is unique to every stem cell type. The critical regulatory components within the niche include dynamic and complex interactions between cells, macromolecules of extracellular matrix (ECM), biochemical components such as signaling molecules and hormones, and biophysical components such as ECM stiffness, pressure, shear fluid flow, stress, and strain (Pelham and Wang 1997; Vining and Mooney 2017). While the role of biochemical factors is well established, recent scientific literature points to evidence which indicates that the mechanical and biophysical signals generated from the extracellular milieu affect stem cell proliferation and differentiation (Gerardo et al. 2019; Gungordu et al. 2019). All cells, including stem cells and cancer cells, respond to mechanical cues. In stem cells, biophysical signaling control stem cell differentiation and self-renewal; and, in cancer cells, these signals lead to tumor invasiveness and metastasis (Lee et al. 2019; Choudhury et al. 2019). All these recent developments have led to the emergence of a new discipline—mechanobiology, which combines physical forces with the biological phenomenon.

The emergence of biomaterials has facilitated to artificially recreate biophysical signals experienced by cells under in vivo conditions. These biomaterials can be employed as a carrier for the transplantation of stem cells or to recruit endogenous progenitor cells at the site to repair and reconstruct damaged tissues or organs. A common hurdle in the use of biomaterials in regenerative medicine is the immune response. After transplantation, the biomaterials are extensively infiltrated by immune cells. These cells facilitate in removing cellular debris caused by injury; however, they can evoke inflammatory responses, which might hinder tissue repair and cell differentiation (Mokarram and Bellamkonda 2014). The development of new strategies has made biomaterials more sophisticated with respect to

biocompatibility, biological cues, and the potential to reduce damage by an immune response and facilitate in vivo tissue development and direct repair.

In this chapter, we have explored the mechanical and functional interactions between stem cells and their microenvironment. We begin with a brief overview of the importance of ECM in mechanobiology, along with the fundamental molecular mechanisms and the emerging field of biomaterials for stem cell culture. We touch upon cancer mechanobiology and the implications of stem cell mechanobiology and regenerative medicine. We finally provide a perspective on the use of biomaterials to create a modified 3D microenvironment for stem cell culture, which will provide a model to uncover fundamental aspects of mechanobiology and hold tremendous potential in cell-based therapies.

15.2 Unique Tissue-Specific ECM Stiffness in Normal Physiology

The ECM is composed of fibrous proteins such as collagen, fibronectin, elastin, vitronectin, laminin; proteoglycans, and glycoproteins secreted by cells and matricellular-associated proteins such as CNN family, osteopontin, fibulin, periostin, and secreted protein acidic and rich in cysteine (SPARC); however, the ratios of these proteins vary between tissues (Yue 2014; Mouw et al. 2014). Therefore, each tissue has different stiffness, which is defined as elasticity or Young's modulus (E) and is measured in a unit called pascal (Pa). For instance, bone ECM is primarily made up of collagen, which makes it stiff, and the estimated stiffness is approximately within the range of 100 kilopascal (kPa)–1 gigapascal (GPa). On the other hand, brain ECM has low fibrous proteins and higher amounts of proteoglycans compared to bone with E of approximately 1 kPa (Fig. 15.1) (Ruoslahti 1996; Wells

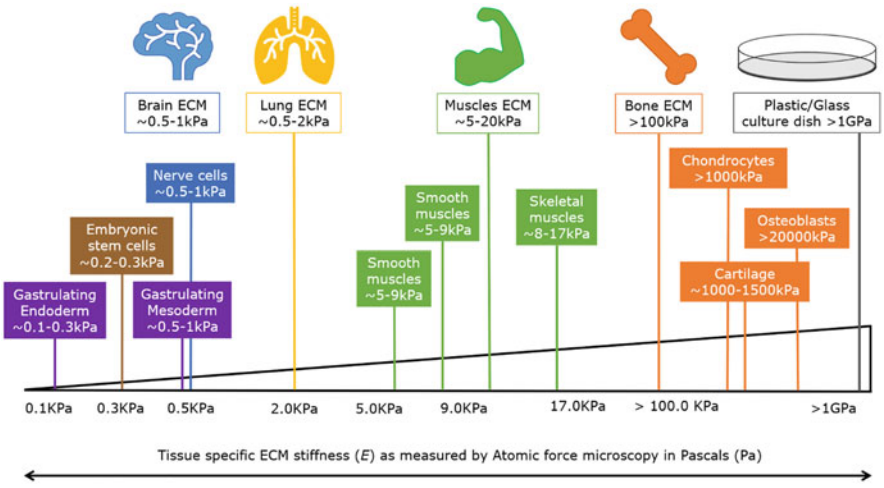

Fig. 15.1 Diagrammatic representation of the varied ECM stiffness range reported in different tissues measured by atomic force microscopy (AFM). The stiffness is defined in Young's modulus or elastic modulus (E) and measured in pascals (Pa)

2008; Budday et al. 2015). Such variations in tissue ECM have led researchers to develop scaffolds that mimic the biological ECM stiffness and properties.

Our understanding of how mechanical signals direct molecular signaling during embryo development and in in vitro differentiation is constantly evolving. The role of ECM in generating mechanical cues has been explored extensively, as the matrix is crucial in regulating cellular functions (Pelham and Wang 1997; Vining and Mooney 2017). Other than providing physical support for growth attachment, the ECM also regulates cell shape, growth, proliferation, differentiation, and migration. Numerous studies have reported that changing the mechanical properties of the matrix, such as stiffness, affects cell morphology, growth, differentiation, migration, and gene expression (Pelham and Wang 1997; Lo et al. 2000; Justin and Engler 2011; Toh et al. 2012, Ireland and Simmons 2015).

15.3 Biomaterials and Their Types

Traditionally used synthetic scaffolds from 2D polystyrene surfaces to 3D constructs provide only support to the cultured cells. Recent advances in tissue engineering have shown exciting results with various biomaterials of suitable physical and chemical properties in recreating complex in vivo microenvironment in the laboratory. Based on their source and properties, these biocompatible materials can be categorized as natural, semisynthetic, and synthetic biomaterials, with stiffness similar to the stiffness of the biological tissue (Virdi and Pethe 2021).

Natural biomaterials are synthesized using polymers such as chitin, agarose, collagen (Chevallay and Herbage 2000), alginate, and hyaluronic acid hydrogel (Toh et al. 2012) because of their similarity with native ECM. Another advantage is that they are highly biocompatible with binding sites for cells, thereby supporting cell growth. However, natural polymers are not consistent in composition, are not easy to modify, and have limited mechanical properties. To overcome these disadvantages of natural polymers, synthetic substrates have been synthesized using polyacrylamide (PA) gels (Engler et al. 2004), polydimethylsiloxane (PDMS) (Goffin et al. 2006), polyethylene glycol (PEG) hydrogel (Gilbert et al. 2010), and polyvinyl alcohol (Muduli et al. 2017), which provide better mechanical properties than natural biomaterials. The synthetic biomaterials provide a range of various stiffness similar to the stiffness of the biological tissue, have high reproducibility, and are well defined. However, synthetic polymers provide limited cell-ECM interactions as they lack the functional group to allow cells to attach.

To overcome the drawbacks of natural polymers and synthetic biomaterials, a semisynthetic hydrogel, for example, gelatin methyl acrylate (GelMa) (Guilak et al. 2009), was designed, which has the biocompatibility of natural polymer and mechanical properties of synthetic biomaterials. To enhance the clinical application of scaffolds, it is important to achieve a xeno-free, chemically-defined system for stem cell culture other than hydrogels. In this regard, other scaffolds such as artificial nano- and micro-patterned substrates (Théry 2010), flexible micropillars (Halder et al. 2012), and electrospun nanofibers (Maldonado et al. 2015; Zhu et al. 2019)

have been synthesized to study the effect of substrate stiffness on stem cell growth, differentiation, and migration.

15.4 Immunomodulatory Biomaterials

As we have introduced above, biomaterials being a foreign material may provoke an immune response, which might hinder tissue repair and regeneration. To address this limitation, researchers are synthesizing new biomaterial designs, which incorporate immunosuppressive molecules or signaling molecules that facilitate activation of the desired phenotype within the host immune cells (Dziki and Badylak 2018). These types of biomaterials are known as immunomodulatory biomaterials. Specific and durable immunomodulation can be achieved by manipulating the surface property of the biomaterials such as topology, surface charge, and ligands; this can induce activation of a desired immune cell phenotype (Stabler et al. 2019). For instance, following the implantation in murine subcutaneous implant and volumetric muscle injury model, flow cytometry analysis identified macrophages ($F4/80^+$), $CD11c^+$ dendritic cells, $CD3^+$ T cells, and $CD19^+$ B cells within the microenvironment of the ECM bioscaffold (Sadtler et al. 2017). The authors have shown that the biomaterial microenvironment changes the polarization of the migrating immune cells upon implantation, causing them to alter the signals generated by microenvironment. This immunomodulatory effect of the biomaterial on the immune cells and the host tissue environment may help in improving the therapeutic capability of the biomaterials. Numerous similar studies that use ECM-based biomaterials show a dynamic interaction between a variety of the immune cells or between stem cells and immune cells, which promotes tissue repair (Brown et al. 2012; Sadtler et al. 2016; Dziki et al. 2018).

15.5 Biomaterials Influence Stem Cell Proliferation and Functionality

In order to design the biomaterial that allows stem cells to be transplanted for clinical use, it is important to study some key aspects such as (1) the traction forces exerted by the cells on the biomaterial, (2) stem cell growth and proliferation, and (3) the changes in the stem cell functionality and differentiation capacity when grown on biomaterial.

The synthetic hydrogel substrates are synthesized using one or more polymers, which forms an interconnecting network with the help of a cross-linking agent. The mechanical properties such as hydrogel substrates can be manipulated by changing concentrations of polymer and cross-linking agent. For example, in PA-gel substrates, altering the ratio of acrylamide to bis-acrylamide cross-linker allows variation in Young's modulus, which thereby affects cell behavior (Tse and Engler 2010). Human mesenchymal stem cells (hMSCs) cultured on stiff PA substrate with $E \sim 25$–40 kPa, which resembles bone ECM stiffness, differentiate toward osteoblast

lineage as indicated by the gene expression analysis, whereas, on soft PA substrate ($E \sim 0.1$–1 kPa) resembling brain ECM stiffness, the hMSCs differentiate toward neural lineage (García and Reyes 2005; Engler et al. 2006). Muscle stem cells self-renew when cultured on substrates mimicking the stiffness of muscle tissue ($E \sim 12$ kPa), and these cells contributed to muscle regeneration when transplanted in mice (Gilbert et al. 2010). Morphologically, stem cells appear flattened on the stiff substrate and spherical with reduced spreading and stress fiber formation on soft substrate (Deroanne et al. 2001; Engler et al. 2004). These studies reveal varying responses of stem cells toward their microenvironment, and substrate stiffness indicates an important role of substrate matrix in regulating cell behavior.

PA-gel substrate functionalized with glycosaminoglycan (GAG) peptides shows better cell attachment. Following this observation, the research group demonstrated that stiff PA-GAG substrate ($E \sim 10$ kPa) promotes pluripotency of human ESCs as evidently observed from the expression levels of pluripotency marker proteins octamer-binding transcription factor-4 (OCT4) and stage-specific embryonic antigen-4 (SSEA4) (Musah et al. 2012); however soft substrate ($E \sim 0.7$ kPa) selectively differentiated stem cells toward neuronal lineage. The same research group noted an interesting observation that even in the absence of neuronal inducing factor, hPSCs grown on softer substrate appeared neuronal-like phenotype and expressed high levels of tubulin beta 3 chain (TUJ1) protein, a neuronal specific-marker (Musah et al. 2014). A similar observation was reported by another group that used other biomaterials as well of different stiffness (Chen et al. 2020). These studies indicate that substrate stiffness alone can influence hPSC differentiation when cultured with an optimal mechanical microenvironment, independent of soluble signaling factors. Therefore, it can be said that the mechanical signals have a profound contribution on early embryo development and differentiation.

As explained above, when mimicking various physiological stiffness like neural ($E \sim 1$ kPa), muscle ($E \sim 12$ kPa), and bone ($E \sim 30$ kPa) tissues, substrates can induce respective lineage-specific differentiation of MSCs. In addition to cellular function, substrate stiffness also influence cell migration. Cell migration is important in numerous physiological processes such as wound healing, organogenesis, immune response, tumor metastasis, and morphogenesis; thus, it is crucial in regeneration tissue engineering and cancer therapy. Many studies have demonstrated stem cells migrate toward the stiff substrate, whereas neurons show a preference for softer regions (Tse and Engler 2010; Vincent et al. 2013; Flanagan et al. 2002; Hadden et al. 2017). The mechanical properties of the ECM influence the factors known to regulate cell migration, such as the integrin-cytoskeletal interaction and cytoskeletal stiffness. The cells sense the change in the matrix through an active tactile exploration mechanism and respond by exerting contractile forces (Lo et al. 2000). To understand the migration of stem cells on matrix stiffness, MSCs were treated with focal adhesion kinases (FAK) inhibitor and siRNA targeting transcriptional factor Yes-associated protein (YAP) gene. They observed reduced cellular motility of treated cells compared to untreated cells, indicating that FAK and YAP control the movement of cells from the soft region toward the stiff region (Wang et al. 2001; Hadden et al. 2017; Lachowski et al. 2018).

15.6 Mechanobiology: Mechanism of Interactions (Molecular Mechanisms)

Mechanobiology is the study of the relationship between a cell and its microenvironment. The interactions between the cell and the microenvironment mainly occur at the interface. The properties of biomaterials such as hydrophilicity, surface charge, roughness, softness, and chemical composition affect the transplantation success. To improve the interaction between cell and scaffold, the physical, chemical, and biological properties of the biomaterials need to be optimized according to the cell type. Before seeding the cells onto a scaffold, surface modification is necessary to facilitate cell adhesion and growth. Surface modification can be either coating the surface with extracellular membrane protein or modifying the surface using functional moieties, hydrophobic or hydrophilic molecules (Shi et al. 2015; Elosegui-Artola et al. 2017).

A cell senses its external environment via membrane-bound receptors, focal adhesions to the ECM, adhesion junctions between neighboring cells, and gap junctions. The perturbation of protein conformation by mechanical forces influences the cytoskeletal organization, which triggers a series of intracellular signaling pathway resulting in inactivation or inhibition of gene expression, morphology, and motility (Discher et al. 2005; Guilak et al. 2009). Integrin-based adhesion complexes are one of the key molecular players closely associated with actin filaments. Focal adhesion complex, Ras homologous (Rho) GTPases, myosin light chain kinases, and Rho-associated kinases (ROCK) form a link between integrins and actin filaments. The activated focal adhesion complex comprises talin, vinculin, paxillin, alpha-actinin, p130cas, FAK, and SRC formed near cell surface integrin receptor (Geiger et al. 2009). The cells are able to sense the substrate stiffness, topology, surface area, and dimensionality of the scaffold by means of integrin molecules and focal adhesion complexes (del Rio et al. 2009; Amano et al. 2010; Donato et al. 2010; Ciobanasu et al. 2013; Janoštiak et al. 2014; Elosegui-Artola et al. 2017).

In brief, integrins are transmembrane ECM proteins and mechanoreceptors as they sense the change in the ECM, thereby mediating the mechanotransduction by focal adhesions, which link integrins to cytoskeleton (Hynes 2020). A traction force is generated in the actin cytoskeleton, which activates the downstream signaling and translocates the signal into the nucleus. These traction forces are also exerted on the integrins and focal adhesions, thus maintaining them in the isometric tension (Bershadsky et al. 2003). External stresses generate a mechanics-based positive feedback loop by increasing tension on the cell surface receptor and activating G protein Rho and its target ROCK. Stiff substrate results in an increase in kinase activities of ROCK, FAK, and extracellular signal-related kinases (ERK1/2), causing osteogenic differentiation of MSCs. Inhibition of ROCK and FAK leads to downregulation of osteogenic markers during osteogenic induction (Shih et al. 2011). Taken together, this implies that stiff substrates affect the regulation of

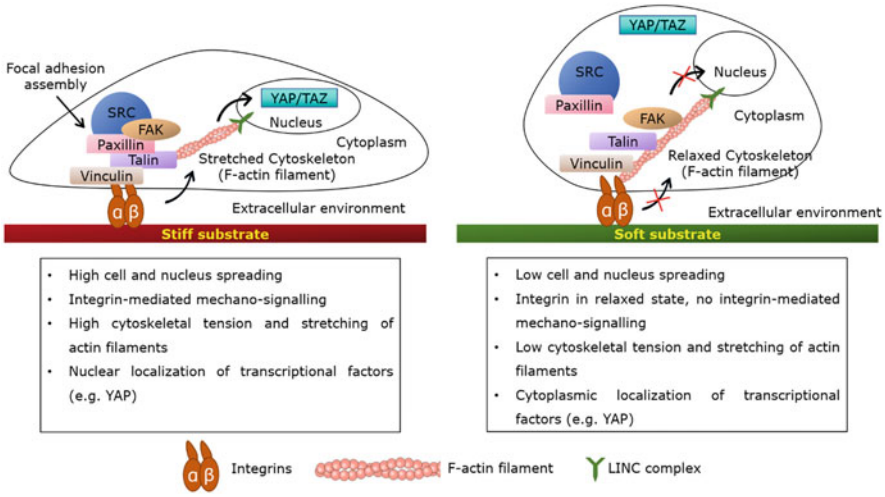

Fig. 15.2 Schematic representation of the effect of stiff and soft substrate on cell morphology and function via integrin-mediated mechano-signaling. On stiff substrate, a cell receives biophysical cues from integrin-based focal adhesion complex, which increases the cytoskeletal stress via stretching of F-actin filaments. The stretching of LINC complex due to stiff substrate and stretched F-actin causes nuclear localization of transcriptional factors such as YAP. Conversely, on the soft substrate in the absence of less integrin activity, the focal adhesion complex is not formed, leading to less cytoskeletal tension and less stretching of actin filaments, thereby leading to cytoplasmic localization and no substrate-dependent nuclear localization of the transcriptional factors

ROCK-mediated FAK and ERK1/2, and these signals regulate the transcriptional factors, thereby determining the fate of MSCs.

The mechano-sensitive transcriptional coactivators such as myocardin-related transcription factor (MRTF) (Speight et al. 2016), nuclear factor kappa B (NF-κB) (Kumar and Boriek 2003), nuclear factor erythroid 2-related factor 2 (NRF2) (Escoll et al. 2020), YAP, and beta-catenin (Gumbiner 1995; Huber et al. 1996) bind to their respective DNA-binding proteins and activate specific genes. The nuclear or cytoplasmic localization of these transcriptional factors is controlled by nuclear envelope receptor—linker of nucleoskeleton and cytoskeleton (LINC) complex (Guilluy et al. 2014; Driscoll et al. 2015) (Fig. 15.2). Apart from integrin-ligand binding, several studies have suggested that the cells produce nano-length projections that sense the surface for optimum spreading. Thus, different nano-topographical features guide cell migration and spreading on the scaffold with different topographies. The fact that cellular orientation and alignment can be controlled by topographical cues was demonstrated as early as 1911 by Robert Harrison (1911). To date, the biomaterial-based scaffold has undergone many surface modifications and alternations and has emerged as a powerful tool for mimicking in vivo microenvironment.

15.7 Biomaterials as Promising Tools for Tissue Engineering and Regenerative Medicine

From the aforementioned considerations, it can be evident that mechanobiological processes in stem cells will impact the development of innovative therapeutic methods for tissue engineering and, eventually, regenerative medicine applications. The successful outcome of any stem cell-based regenerative medicine critically depends on cell survival after transplantation and to maintain tissue homeostasis mainly by differentiating into the respective lineage. To attain this, it is crucial to maintain optimal physiologically similar culture conditions in vitro for stem cell maintenance, proliferation, and quick differentiation when required. For instance, culturing the resident liver stem cells (RLSC) on polyacrylamide gel substrate having a stiffness of 0.4 kPa has shown to help in differentiation of RLSC into hepatocytes within 24 h, whereas RLSC cultured on a stiff substrate of stiffness 80 kPa resulted in only initial hepatocyte-specific transcriptional activity (Cozzolino et al. 2016). This variation in differentiation potential is due to culturing cells on soft stiffness—which is similar to healthy liver tissue stiffness (0.3–6 kPa)—rather than using normal stiff TCP. Similarly, instead of 2D culture system, Schoonjans and colleagues developed a synthetic 3D culture system using polyethylene glycol (PEG) hydrogels with a matrix stiffness of 1.3 kPa. This 3D culture system mimicking physiological liver stiffness provided better efficiency of live organoid derivation from mouse and human hepatic progenitors (Sorrentino et al. 2020). These studies show that clinically relevant human progenitor/stem cells cultured in physiologically relevant mechanical environments open perspectives for liver organoid-based clinical applications.

An interesting study focused on regenerating complex neural tissue such as motor neurons through modulating substrate stiffness because during embryo development, biophysical cues from the surrounding microenvironment along with soluble morphogens like sonic hedgehog (SHH) and retinoic acid (RA) play an important role in morphogenesis. Sun et al. (2014) and colleagues synthesized a system with PDMS with a stiffness range of $E = 1.0$–1200 kPa for generating motor neurons (MN) derived from hPSCs. Their findings suggest that soft substrate ($E = 1$ kPa) support early MN differentiation of hPSCs compared to stiff substrate ($E = 1200$ kPa). In addition, the yield and purity of functional MNs improve four- to tenfold on soft substrate compared to stiff substrate (Sun et al. 2014). Thus, culturing hPSCs on a synthetic cell culture surface with controlled mechanical properties (such as substrate stiffness) improved the efficiency of hPSC differentiation into motor neurons. Such advances open new doors in the therapeutics of motor neuron-associated neurodegenerative (Sun and Fu 2014).

An electrospun nanofibrous vascular scaffold made up of poly(L-lactide) (PLLA) was embedded within PA hydrogel on the outer surface. This nanofibrous polymer system had stiffer matrix near the polymer and was less stiff away from the polymer and was used as a graft for cell regeneration in vivo. Multipotent neural crest stem cells (NCSCs) generated from hiPSCs were embedded within the graft and implanted in rat carotid arteries. The stiffer matrix of the polymer scaffold with

$E = 50$ kPa or higher supported the differentiation of NCSCs into smooth muscle cells (SMCs). The soft matrix area of the scaffold with $E = 15$ kPa supported the differentiation into glial cells. The results suggests that the mechanical properties of substrate play a significant role in designing biomaterials for tissue engineering (Zhu et al. 2019).

hiPSCs are traditionally generated by genetic reprogramming of adult somatic cells using biochemical signals (Takahashi and Yamanaka 2006). Fascinatingly, Grãos and colleagues demonstrate that MSCs can be reprogrammed into iPSCs by biophysical cues alone. They showed that human umbilical cord MSCs (huMSCs) exhibit PSC phenotype when cultured on soft PDMS substrate with $E = 1.5$ kPa and 15 kPa compared to stiff TCP ($E \sim 1$ GPa). huMSCs undergo chromatin modeling and show enhanced expression of pluripotency-related markers *OCT4*, *SOX2*, and *NANOG* in response to the soft substrate. Soft substrate allowed huMSCs to acquire relaxed nuclei, small FA, fewer stress fibers, and high euchromatic and lower heterochromatic content and expression of pluripotency specific genes. In short, their results suggest that substrate stiffness influences several phenotypic features of iPSCs and colonies and that soft substrate favors iPSC reprogramming (Gerardo et al. 2019). Such milestone studies indicate that substrate stiffness is a critical biophysical cue that influences stem cell differentiation into the specific lineage. Such studies also highlight the importance of biomaterials in tissue engineering and a promising platform for improving tissue engineering and regenerative applications.

15.8 Mechanobiology in Cancer Cells

Mechanobiology is one of the driving forces in guiding cell motility and tissue development during embryonic development. This cellular and tissue mechanobiology approach has been used by many researchers in understanding cancer development and tumor invasion. One of the key mechanisms by which cancer cells evade therapy is metastasis, and it has been hypothesized that the tumor cells might rely on mechanical forces for invasion and migration. The tumor microenvironment is an aggregation of cancer-associated fibroblasts (CAFs), vascular cells, immune cells, an abundance of extracellular matrix proteins, and hypoxic conditions (Choudhury et al. 2019; Sahai et al. 2020). Hypoxia and hypervascularization are directly and indirectly associated with ECM realignment and shear stress (Wang et al. 2017).

The ECM is a fundamentally essential component of the tumor microenvironment that interacts closely with cancer cells. Apart from providing necessary growth factors for tumor growth (Briquez et al. 2015), the ECM also helps in transmitting signals integrins (Canel et al. 2013). Additionally, upregulation of ECM remodeling molecules, such as transforming growth factor-beta (TGF-β), is linked to the development of desmoplasia in tumors (Papageorgis and Stylianopoulos 2015). Desmoplasia is the development of dense fibrous and connective tissue around tumor growth, usually characterized by increased synthesis of total collagen, fibronectin, glycoproteins, mainly tenascin C, proteoglycans, and a sizeable stromal cell

population that amasses within the tumor. The increased production of tumorigenic and inflammatory growth factors transforms a large number of fibroblasts into CAFs. It has been proposed that the multifunctional cytokine TGF-β activates the transformation of fibroblasts into CAFs, which produces more ECM fibers, eventually causing desmoplasia (Papageorgis and Stylianopoulos 2015). The ECM stiffness of the fibrotic/cancer tissue is around 1.08–68 kPa (Kawano et al. 2015) and has shown to upregulate alpha-smooth muscle actin (α-SMA) expression, a proven CAF marker. Another known transcriptional factor that facilitates CAF generation and maintenance is YAP/TAZ, which activates only during high actomyosin contractility and high stiffness (Goffin et al. 2006; Calvo et al. 2013). YAP has been shown to regulate the expression of specific cytoskeletal proteins, including anillin actin-binding protein, myosin regulatory light polypeptide 9, and diaphanous related formin 3, which induces CAF (Calvo et al. 2013).

During cancer progression, uncontrolled cell proliferation results in an increase in tumor mass. This leads to a difference between the ECM stiffness of tumorous tissue and normal tissue. For instance, Samani et al. (2007) reported that the mean Young's modulus of normal breast tissue is 1.9 kPa, whereas that of fibroadenoma was 11.42 kPa and that of invasive ductal carcinoma was 22.55 kPa. Multiple in vitro reports show that the stiffness of the tumor tissue and matrix directly correlates with tumorigenesis and metastasis (Zaman et al. 2006; Tilghman et al. 2010; Gkretsi and Stylianopoulos 2018; Jang et al. 2020). A breakthrough study published by Weaver and colleagues proves the hypothesis that mechanical signals mediate malignant transformation. They showed that culturing non-tumorigenic mammary epithelial cells on stiffness mimicking tumor-like stiffness induces cell proliferation, dysplasia and activates oncogenic epithelial signaling pathways. They also found that transformed cells maintain a functional link between integrins and Rho-dependent cytoskeletal tension, and in the presence of ROCK or integrin adhesion pharmaceutical inhibitors the malignant behavior of tumors was tempered (Paszek et al. 2005).

Cancer stem cells (CSCs) have been shown to reside within the tumor, and these cells have the ability to self-renew and differentiate into several cell types, which proliferate uncontrollably. Thus, CSCs sustain the growth of cancerous mass. The cancer stem cells are hard to eliminate due to their efficient DNA repair mechanisms, relative slow growth rate, and the high number of channel proteins to efflux drugs out (Turdo et al. 2019; Hirschmann et al. 2004; Fujiwara et al. 2021). Cancer stem cells lead to relapse of cancers after treatment (Eyler et al. 2008), and hence, it is necessary to investigate these cells including their mechanobiology machinery. In summary, understanding how cancer cells sense the mechanical signals and converted them into biochemical pathway may usher in new ways to control cancers. Given the similarities between the biology of stem cells and cancer cells (Shackleton 2010; Rahman et al. 2016), researchers are exploring the functional and mechanistic similarities between stem cell mechanobiology and cancer mechanobiology, with the aim of understanding the former using the latter as a guide (Fig. 15.3).

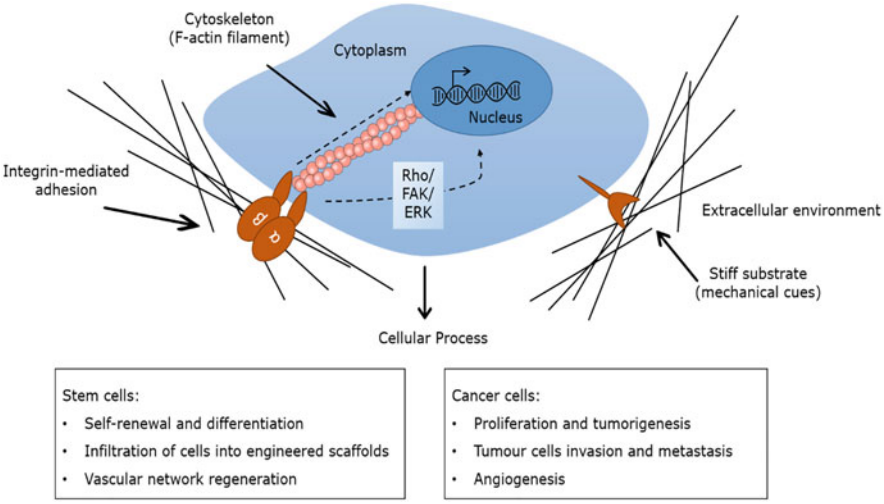

Fig. 15.3 The similarities between the ECM-cell mechanobiology of stem cells and cancer cells

15.9 Concluding Remarks and Perspectives

Many advances in fabricating biomaterials for regenerative medicine have been reported in recent decades. Fundamental properties of biomaterials and of cell responses to biochemical and biophysical cues have been described via structural and functional studies. In this chapter, we have briefly described various properties of biomaterials and their impact on cellular behavior. For detailed information on the physical, chemical, and functional properties of the biomaterials, the authors recommend some extensively detailed reviews by Amani et al. (2019) and Cun and Hosta-Rigau (2020). The existing knowledge on ECM-cell interactions has been mainly derived from 2D in vitro studies. Although the 2D culturing system is convenient and has uncovered several crucial aspects about mechanobiology and biomaterials in cell migration, adhesion, proliferation, and differentiation, it does not mimic the in vivo microenvironment, which is 3D. It is becoming increasingly evident that the cells have a distinct behavior in the 3D microenvironment than that seen in 2D microenvironment. These facts have led to the use of a 3D culture system to mimic the physiological environment required for stem cell differentiation and the generation of organoids (Pepelanova et al. 2018; Bailey et al. 2019). hPSCs cultured on 3D scaffold have already been used to develop neuronal (Levenberg et al. 2003), liver (Baharvand et al. 2004), and cartilage (Hwang et al. 2006; Bai et al. 2010) tissue equivalents, along with rudimentary vascular networks (Ferreira et al. 2007).

Other than 3D culture, 3D bioprinting can be used to fabricate well-organized cell-laden scaffolds, which can be used to repair or regenerate damaged tissue

(Antich et al. 2020; Jeong et al. 2020). Further advancement is organ-on-a-chip technology, which helps in generating self-organizing miniature organs from stem cells that replicate the functional and structural characteristics of cells present in in vivo microenvironment (Park et al. 2019). This organ-on-a-chip method has been employed in cancer cells to understand the disease progression and predict drug-induced responses (Sun et al. 2019). The studies discussed herein demonstrate the significance of the extracellular microenvironment in determining cellular behavior. They also highlight the importance of developing novel biomaterials to provide cells with biophysical cues which will help in cell-based therapies and regenerative medicine. Although much is yet to be unraveled about the influence of mechanobiology on stem cells, the newer discoveries give us insight into a promising future but also raised certain fundamental questions, such as the following: How much of the mechanical information is needed for the desired response from stem cells to form complex tissues? Can the biomaterials transplanted cause uncontrolled proliferation of the surrounding tissue? How cells generate their own mechanical forces during embryogenesis? With such diverse materials and methods for synthesizing biomaterials, it becomes crucial to understand how much of the material complexity is required for the desired stem cell response. We envision that the current research will help pave the way in understanding mechanobiological influence on stem cells and have major implications on tissue engineering and regeneration approaches.

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