Chapter 7

Mechanical Stimulation of Cells Through Scaffold Design for Tissue Engineering

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Abstract

Tissue engineering scaffolds attempt to mimic the stem cell environment by creating different biophysical and chemical signals. On the other hand, stem cells are able to sense these characteristics and change their destiny. Scientists try to explain these phenomena through scaffold design and *in vitro* experiments, but the mechanisms implicated remain unclear. Moreover, environment-cell interactions are a key process to get organs and tissues arrangement. Therefore, this chapter deals with the mechanical signals and mechanism involved in cell behaviour through scaffolds as a strategy in tissue engineering.

Keywords: stem cells, extracellular matrix, tissue engineering, scaffold, mechanical cues

1. Introduction

It has been more than 300 years since Robert Hooke first observed a cell and more than 150 years that the cell theory was postulated. Although all living organisms are made up of cells, not all cells are the same. There is a great variety in their shape and most importantly in their function [1, 3]. Different aspects have been revealed about how cells communicate, differentiate and respond to certain stimuli. Nevertheless, the answers remain incomplete and cell responses can be catalogued as dynamic and complex.

Individual cells sense and respond to the environment at different levels (micro- and nanoscale). In multicellular organisms, cells interpret signals of the micro-environment and

neighbouring cells. All these signals end up coordinating the growth and development of organs and tissues [1]. According to Bissell et al. [2], the function of a tissue is regulated reciprocally and dynamically. The micro-environment is formed by the extracellular matrix (ECM) and is able to send signals to the cells. These signals can reach the nucleus and direct the cellular behaviour. The above is supported by the theory called 'dynamic reciprocity'. These concepts are important for tissue engineering, which is defined as a multidisciplinary science that applies principles of engineering and life sciences to the development of biological substitutes that restore, maintain and improve the functions of tissues. The concept that cellular behaviour can be directed by modifying cell micro-environments implies that biomaterials need to build and imitate the ECM. Since each cell resides in a different micro-environment, the biomaterials or scaffolds should be available with precise characteristics [3].

In particular, mechanical forces in cell behaviour have only recently begun to receive attention. For example, mechanical overloading can induce deformation and remodelling of cells, which significantly affects the cellular function. Also, living cells support or create forces; mechanical loading induces deformation and remodelling, which influence many aspects of human health and disease.

Therefore, more importance has been given to stress in cell behaviour [4]. Modelling the constitutive behaviour of cells through biophysical signals poses a challenge. The stimuli reside *in vivo*, but the challenge is mimicking the properties *in vitro* [5]. Imitating stem cell biophysical niches with biomaterials could facilitate the production of large numbers of stem cells needed for *in vitro* regenerative medicine. In recent years, researchers have tried to evaluate the significance of physical cues that influence stem cells; such as stiffness of cell culture substrates and other applied mechanical forces [6]. Several studies explore the regulation of stem cells via fluid shear stress, hydrostatic pressure, ECM elasticity, substrate topography and tension [5]. However, how cells can sense mechanical forces or deformation and convert them into signals is not well understood. Also, the mechanism and the communication pathways remain unclear.

2. Mechanical properties of natural extracellular matrix

ECM is a macromolecular aggregate where the cells reside, proliferate and perform different functions. Their components are normally produced by cells or provided by bloodstream [7]. ECM can also be defined as secreted molecules (including grown factors, cytokines and cell adhesion molecules) that are immobilized outside the cell. Macromolecules of ECM are collagen, elastic fibres and proteoglycans; they are mainly responsible for tissue-type specific extracellular architecture [7, 8].

Collagen and elastic fibre system constitute the architecture of ECM [7].Collagen is a large family of molecules having the ability to aggregate making a supramolecular structure. It is composed of three polypeptide chains forming a triple helix. Elastic fibres are assembled by elastin, an insoluble polymer. Additionally, glycoproteins act as adhesion molecules of intercellular substrates, which are very important in cell-cell and matrix-cell interactions.

Basically, proteoglycans are macromolecules covalently associated between polypeptide chains and glycosaminoglycans [8]. They contribute to cellular adhesion through interaction between matrix components and cellular surface [8, 9]. Principal multifunctional glycoproteins include: laminin, fibronectin, tenascins and thrombospondin. They interact with other molecules, such as integrins, cadherins, immunoglobulins and selectins, and serve as a union between ECM and cytoskeleton [8].

ECM has an important role in regulating the development, function and homeostasis of all eukaryotic cells. This matrix provides physical support for cells and participates in establishment and maintenance of differentiated tissues and organs. Also, it regulates the presence of growth factors and receptors, the level of hydration and pH of the local environment [8, 9]. Interactions between cells and the environment (i.e. ECM) are important in processes such as development, homeostasis and pathogenesis [9]. ECM composition and topography are generated through a dynamic biochemical and biophysical interplay between the various cells in each tissue. The mature ECM can also undergo dynamic remodelling in response to environmental stimuli, such as applied force injury, which enables the tissue to maintain homeostasis [10].

Biophysical considerations of native ECM include the mechanical properties, which vary depending on the tissue. For example, animal connective tissues (tendons and the dermis of the skin) can be rough and flexible, or hard and dense like bone. The range of elasticity in tissues is very wide (~0.1 kPa to 20 GPa) (**Table 1**). For instance, the variation in stiffness can have deep effect in cells (spreading, migration, signalling, differentiation and tumour formation) [7, 8]. Differences in the mechanical properties of tissues may depend on the presence of a disease process or the age. For example, while normal breast tissue has an elastic modulus on the order of 1.2 kPa, breast tumours are significantly stiffer (2.4–4.8 kPa). Another example is the progression of carcinomas, where matrix stiffness increases due to an increased deposition of collagen [8].

The theory of tensegrity states that there is a balance of compression and tension. In this context, the elements resist compression and bring the system into a self-sustained state that maintains size and form. The cytoskeleton is a complex structure that supports and responds to mechanical forces and changes depending on the extracellular forces or conformational alterations in the membrane. Forces can be transmitted, due to modifications, directly to the

Tissue	Elastic modulus (kPa)
Pre calcified bone [7]	~80
Trabecular bone [7]	2×10^7
Muscle [7, 9]	~10–13
Brain [7, 9]	~0.2–1
Adipose [9, 11]	~2–4

Table 1. Elastic modulus of different tissues in human body.

cell nucleus and alter shape, rearrange centromeres and modify gene transcription. Therefore, structural alterations and remodelling of the cytoskeleton in response to mechanical forces might be essential in mechanosensing and cell behaviour. Moreover, stem cells can change from quiescence into differentiated cells in response to biophysical signals such as mechanical forces [5, 6].

Force application to a single element results in the distribution of forces and rearrangement of elements that can span across long distances and different scales. Actomyosin filaments can generate tensions, which are driven by molecular motors that convert the chemical energy of adenosine triphosphate into mechanical forces [5]. Different cytoskeletal filament systems are interconnected with each other. Therefore, a tensile stress is generated in cytoskeleton through a balance between opposing forces. Depending on the level of the tensile stress, cell stiffness increase in a proportional manner, this is called prestress. The prestress in cells can be elevated internally by stimulating actomyosin-based contraction, disrupting microtubule compression struts, or externally increasing the ability of the ECM or other cells to resist contractile forces.

Living cytoskeleton is stabilized by a tensile prestress that is generated and maintained through a force balance between contractile actomyosin filaments. Actin cytoskeleton has a prestress transmitted by traction forces that act at cell-anchoring points. There is a coupling between the cytoskeletal contractile actin network and microtubules analogue to tension-compression coupling in tensegrity structures. Stiffening in living cells is mainly due to geometrical rearrangements, bending or buckling of the structures of the cytoskeleton [6, 12].

Overall, mechanical forces play a central role in understanding how biological patterns and morphologies emerge and vary along evolution. In multicellular organisms, tensional forces applied by cells to the ECM are balanced by equal and opposite forces. Stress is defined as force per unit area. Several studies explore the regulation of stem cells via fluid shear stress, hydrostatic pressure, extracellular matrix (ECM) elasticity, substrate topography and tension [5, 11].

There is a challenge in the characterization of the mechanical properties of natural ECM that arise from their complexity and dynamic nature. For instance, the heterogeneous characteristics of ECM complicate the task. Also, the variability of a biological structure depends on several factors (i.e. tissue type, age, etc.). Simple methods used to measure mechanical properties are those based on the analysis of deformations without association with actual forces. More sophisticated methods include the use of tools such as atomic force microscopy (AFM) [13]. For example, Wu et al. [14] described a protocol to measure the membrane plasticity and mechanical dynamics of individual hippocampus neurons in a murine epilepsy model with AFM.

3. Cell matrix interactions

Cells are surrounded by ECM and are responsible for its composition, structure and mechanical properties. For example, fibroblasts build the ECM in soft connective tissues. At the same time, ECM is fundamental in many cellular processes (spreading, migration, proliferation and differentiation) and tissue functions [15]. Therefore, a type of communication

is generated between cells and ECM. In the tissues, cells adhere to ECM or to nearby cells through the formation of (cell-matrix and cell-cell). These junctions allow a transmission of signals (i.e. mechanical) among the different biological structures (**Table 2**) [16, 12].

Cell-ECM interactions are mediated by an integrin family of migration-promoting receptors that interact with the actin cytoskeleton in the cell. The integrins are heterodimeric receptors consisting of α and β chains with large ligand-binding extracellular domains and short cytoplasmic domains. Humans have at least 24 different kinds of integrins, which recognize different extracellular structures and have distinct functions depending on the type of cell in which they reside. Recent studies have identified a set of integrin-associated cytoplasmic proteins such as talin, vinculin and p130Cas [13, 18, 19]. Integrins themselves do not have catalytic activity and do not initiate signalling cascades; therefore, signals are transmitted through direct and indirect interactions with several integrins. Nowadays, there are many questions related to the molecular mechanisms mediated by integrins, although it is clear that integrin-mediated adhesions can sense the properties and characteristics of the ECM [14].

On the other hand, actin is an abundant protein, which can be found in globular (G-actin) or filamentous (F-actin) form. Actin proteins are well known as an essential component of the cytoskeleton. Cells have an actin layer coating the plasmatic membrane that has a critical role in controlling changes in cell morphology [6, 14]. Integrins and actin are separated by a high focal adhesion core-region consisting of specific protein layers. It is suggested that the first section includes a signalling layer consisting of cytoplasmic tails, focal adhesion kinase (FAK) and paxillin. The second layer is an intermediate stratum related to force transduction it contains talin and vinculin. The third layer is composed of an actin-regulatory surface containing vasodilator-stimulated phosphoprotein, zyxin and α-actinin [6, 20].

Focal adhesions and actin proteins have important functions in various cell-signalling pathways and cell fate. The signalling and mechanosensory system of the adhesions are organized in a nanoscale manner. Focal adhesions are flat and elongated structures often located near the periphery of cells [21].Recent studies have revealed a set of proteins responsible for

Cell-cell	**Associated proteins**
Tight junction	Transmembrane proteins, actin
Adherens junction	Actin micro-filaments
Desmosome	Intermediate filaments
Gap junction	Connexins
Hemidesmosome	Intermediate filaments
Cell-matrix	
Focal adhesion	Actin micro-filaments, integrin
Hemidesmosome	Intermediate filaments, integrin

Table 2. Cell junctions and associated proteins [12, 13].

sensing mechanical force and regulating cell-ECM and cell-cell adhesions. They are a part of the linkage between cytoskeleton and cell adhesions and are subject to tensile forces produced by actomyosin contraction [22]. Biomaterials (synthetic or natural) can modulate the effects of these soluble factors by temporally or spatially controlling their delivery. They promote the organization of focal adhesions. For example, Sequeira et al. [23] investigated the influence of scaffolds surfaces in cell attachment, tissue morphology and formation of focal adhesion complexes. In this study, they used an adult mouse submandibular salivary gland ductal epithelial cell line. It was a relationship between the focal adhesions complexes formed and the type of substrate used. Moreover, cells seeded in nanofibre scaffolds showed the fewest focal adhesion complexes; meanwhile in polymer films were abundant. Focal adhesions complexes are mechanisms for scaffold-cell communication. Therefore, they are important to sense biomaterial cues that can direct their fate.

Several authors have discussed and trying to explain the communication pathways between the cells and the micro-environment. Bissell et al. [24] have shown in previous studies that some pathways can be turned on and change gene or protein expression indicating a dialogue between the components of the tissues.

4. Understanding mechanical stimulation through scaffold design

Mechanotransduction is the process by which physical cues are translated into biochemical signals. This route is mediated by focal adhesions. There are two types of forces that the cells can experience, those applied from the environment and those that the cell generates itself. In response to external forces or other stimuli, cells can produce internal forces either by extending membranes or by rearranging their actin cytoskeleton. In this way, they produce endogenous contractile forces [25]. It has been suggested that mechanical forces applied to proteins may perturb the conformations and expose the hidden binding sites, resulting in mechanical signalling processes [12, 13].

Externally applied forces are detected by numerous cell-surface adhesion receptors, such as integrins and cadherins. The ability of these receptors to respond to external forces directs cell behaviour and tissue homeostasis. The force that is applied to integrins is sensed and supported by cytoplasmic components, which at the same time are capable of generating a response [6, 16]. Forces applied trigger actin cytoskeletal rearrangements, activating the small GTPase RhoA and enhancing the activity of myosin II. Subsequently, contraction forces are generated through actin and myosin II filaments. These events create a response through the association of adhesion complexes and the establishment of an internal force. This process is known as reinforcement or cell stiffening [16].

An important theory has been introduced, *Extracellular Matrix Tethering Hypothesis*. In this case, the cells do not directly sense the bulk stiffness of the underlying substrate; instead, respond to the mechanical feedback presented by covalently anchored ECM molecules such as collagen. The exact sequence of events and molecular mechanisms remain to be unrevealed [9]. Despite this, it has been well established that the mechanical properties of

materials regulate cell behaviour. Although it is unclear how physical properties (i.e. stiffness and viscoelasticity) are capable of controlling different functions in cells, biophysical aspects of the ECM are associated with different cellular actions *in vitro* and *in vivo* [26].

Since biophysical and biochemical properties of native ECMs are difficult to control, synthetic materials are important to recreate mechanical characteristics of ECM. Several studies have analysed cell behaviour depending on different mechanical properties controlled through synthetic substrates. For example, Chaudhuri et al. [19] investigated the influence of hydrogel viscoelasticity and stress relaxation on spreading, proliferation and differentiation of mesenchymal stem cell (MSC). MSC differentiation depended strongly on the initial elastic modulus of 3D hydrogel matrices, with osteogenesis occurring only when the initial elastic modulus was 17 kPa. In this work, an approach to modulate stress relaxation properties in alginate hydrogels was showed and demonstrated that substrate stress relaxation influences cell behaviour [19, 25].

Also, Baker et al. [27] explain mechanisms of how cells interpret ECM stiffness in fibrous networks, which are synthesised by electrospinning and soft lithography and coupled with RGD peptides. They found that fibrillar topography had a stronger influence on cell morphology than the biochemical nature of these interactions. Moreover, Huebsch et al. [28] studied the response of mouse mesenchymal stem cells (mMSC) seeded on injectable void-forming hydrogels. The morphology of mMSC was initially similar in standard and void-forming hydrogels. But, after void formation, cells neighbouring to pores exhibited extended, spread morphology, whereas cells in standard hydrogels maintained a rounded morphology. Furthermore, Fusco et al. [17] studied the existence of a relationship between substrate stiffness and characteristics of focal adhesions with mouse embryo fibroblast NIH/3T3. They developed two different materials: polydimethylsiloxane and polyacrylamide. Their results suggested that focal adhesions are sensitive to elastic properties of the materials while cell spreading is dependent of substrate viscoelasticity.

Other studies have been focused on techniques to stimulate cultured cells with mechanical cues. Special attention has been given to the bone cell lineage since skeleton is responsible for withstanding load bearing. Techniques such as mechanical compressive forces have been shown a variety of cell responses *in vitro* that include cell proliferation and differentiation. Compressive loading of stem cells, in particular, mesenchymal stem cells, has been studied and related to chondrogenic differentiation. For example, Steinmetz et al. [29] demonstrated that in a dynamic *in vitro* environment, human mesenchymal stem cells (hMSC)seeded on hydrogels showed high expression of collagen (I, II and X). This study suggested that mechanical stimulation has a positive impact on hMSC differentiation. Also, some studies have been focused on the effects of mechanical stimulation on diseases states of cells. For instance, Tse et al. [30] suggested that compressive stress accumulated during tumour growth could enable migration of cancer cells, therefore promoting cancer cell invasion.

The mechanical stimulation *in vitro* has been studied with the addition of molecules that are able to induce expression of genes involved in differentiation processes. Also, some studies demonstrated that mechanical loading is able to induce differentiation of cells without the help of biochemical molecules. For instance, a recent study showed the effects of mechanical strain in mesenchymal stem cells seeded on silicon substrates. In this study,

the mechanical stimulation was the main variable. There was not any addition of chemicals to promote proliferation or differentiation. Their finding suggested that mechanical strain enhances the proliferation of MSCs [31].

Despite several studies have been documented the effects of mechanical properties on cells with the help of synthetic materials, several questions concerning the mechanisms remain unclear. For example, it is not known the specific pathways that regulate the switching between homeostatic and disease states. Moreover, these states are related to the progression from soft to stiff characteristics in tissues.

5. Tissue engineering and scaffold mechanical properties

Tissue engineering is an interesting approach aimed to reconstruct or create new tissues. However, building new tissues is an enormous challenge, for instance, several tissues are composed of different cell populations [32]. An advantage is the self-repair ability of cells that can be used in favour of tissue engineering scientist. However, the poor understanding of cell repair mechanisms and the additional challenges of biomaterial design have been slowed the progress in this area. When some circumstance (age, wound size, inflammation or chronic disease) inhibits the natural repair process, an alternative method to healing is required. Tissue engineering considers the use of biomaterials and cells from autologous or external sources. Basically, biomaterials or scaffolds are aimed to help cells in the proliferation and differentiation processes. Then, biomaterials and cells are the beginning formulae to create a new organ or tissue. However, we have to remember that cells need to get the right instructions to start the process of self-repair. These instructions are delivered through physical or chemical cues included in biomaterials [3, 23].

The inspiration of biomaterial design is the ECM, which properties are crucial for cell behaviour as discussed before. All cells receive signals from ECM, so scientists have attempted to mimic the physical and chemical characteristics [23]. Some scientists have been focused on imitating patterns, forms, textures and specific characteristics such as mechanical resistance and chemical structure. For example, Zhang et al. [33] constructed a three-dimensional system to create tissue architecture. The scaffold systems were synthesised with an elastic modulus similar to brain tissue. Additionally, they encapsulated a laminin protein, which is a neural ECM component. A rapid maturation of neurons from human induced pluripotent stem cells was associated with the physical properties of the scaffold systems, which are similar to the mechanical properties of the natural extracellular matrix in the brain.

On the other hand, stem cells are widely employed in the tissue engineering area due to their potential to give rise to different cell types. Also, stem cell differentiation through biomaterial mechanical properties remains a critical goal [34]. For example, changes in the bulk stiffness of ECM-coated hydrogels elicit different cell responses. In the case of mesenchymal stem cells, bone differentiation is favoured by stiffer substrates, whereas adipocyte differentiation is promoted by softer substrates. The influence of mechanical properties on stem cell differentiation has been demonstrated on a range of substrates, including collagen and hyaluronic acid gels, Poly(D-lactide-co-glycolide acid) electrospun nanofibres and polydimethylsiloxane, among other biomaterials [35]. For example, Shih et al. [31]

studied the mechanisms of osteogenic differentiation from bone marrow mesenchymal stem cells. Polyacrylamide substrates with different young´s modulus were synthesised to analyse secretion of molecules involved in cell differentiation. They found that production of collagen type I increased in cells seeded in stiffer substrates. Also, they demonstrated a higher level of mineralization and a higher FAK and RAK activation (mechanoresponsive elements) when stiffer matrices were used. The expression of integrin was also different depending on the elastic modulus of the biomaterial. For instance, integrin expression per cell was statistically higher on stiffer matrices.

In the same way, Banerjee et al. [36] examined the behaviour of neural stem cells encapsulated in three-dimensional scaffold (alginate hydrogels) with a variable elastic modulus. They analysed the differentiation of cells with neural marker β-tubulin III. Proliferation of cells increased significantly with a decrease in the elastic modulus of hydrogels. The maximum intensity of β-tubulin III staining was observed in cells grown in the hydrogel with the lowest modulus. The modulus ~180 Pa promotes neuronal differentiation which is related to the elasticity of brain tissues. Overall, these results demonstrated the influence of the mechanical characteristics of the biomaterials on cellular behaviour *in vitro*. Moreover, there is a diversity of biomaterial systems that can be used to investigate cellular behaviours to mimic native ECM. Stem cells in these different biomaterials had a diverse behaviour related with mechanical properties of the scaffold and confirm that stiffness is an important factor [37].

In the 1980s, the main function of a biomaterial was limited to support cells. However, as stated above, biomaterials can influence different cellular processes depending of the physical characteristics such as young's modulus [32]. As an example, biomaterial stiffness has been found to affect the transcriptional process [38]. In this context, studies have been shown that certain cell lines develop larger focal adhesions on stiffer surfaces. Also, cell migration speed had showed a dependence on mechanical properties. Other studies had demonstrated that cells migrate preferentially to stiffer surfaces. However, the influence of substrate mechanical properties on cell phenotype also depends on the cell type [23, 38].

The cellular behaviour and mechanical stimuli *in vivo* models have also been analysed. For example, Moshayedi et al. [37] developed a hydrogel material to control neural cells fate *in vivo*. In this study, an injectable hydrogel was designed and showed to promote survival and differentiation towards immature states of human neural progenitor cells. Another study employed magnetically responsive ferrofluid microdroplets to measure local mechanical properties in developing embryos. Their results suggested that tissue mechanics might play a critical role in morphogenesis [39].

6. Additional considerations about cell biomechanics: the case of the adipocyte

As shown in this chapter, cells respond to external environmental forces. Such understanding about cell behaviour would also benefit from studying how cells react to biomechanical disturbances

inside them. In this section, a source of physical strain within the cell is presented. The case is made for the fat overload in adipose cells, a common condition in people with obesity.

Adipocytes are cells specialized in storing triglycerides in the form of lipid droplets [40]. The adipocyte can form one giant lipid droplet as large as 100 μm, this constitutes the most efficient cellular packaging of energy per volume, which is a favourable trait to conserve energy that could be used when energy supply decreases [41]. However, there are factors in modern life such as frequent intake of energy-dense food that contribute to adipocyte hypertrophy [42].

A question is why the adipocyte continues accumulating fat even when it is unhealthy [43]. One possibility is that triglyceride accumulation may be part of adaptive mechanisms that prevent toxicity induced by high levels of lipids [44].

Despite the high resilience of adipose cells to fat overload, excessive accumulation of triglycerides within the adipocyte impairs its cellular functions [45]. For instance, a negative effect of excessive packing of fat by the adipocyte includes induction of cellular hypoxia through inhibition of effective oxygen supply from the circulation [46]. Another negative intracellular effect of adipocyte hypertrophy is a mechanical stress on the endoplasmic reticulum, condition that impairs protein folding [47]. Indeed, the adipocyte displays a potent inflammation as effect of the high storage of fat [48]. The impact of hypertrophy can be so adverse as to trigger adipocyte apoptosis [49]. Nevertheless, before such ultimate death phase occurs, the adipocyte enacts a series of responses to improve its own functioning as fatness accumulation increases in its intracellular space.

It has been proposed that adipocytes contribute to sense the levels of body energy (fat content) and are able to signal such state to the central nervous system that in turn modulates individual's intake and expenditure [41]. Although the somatic influence on appetite seems to be not as strong as needed to reduce overeating behaviour [50], to deal locally with lipid accumulation, the adipocyte increases its metabolic pathway for fat oxidation [51, 52] In addition, the adipocyte signals immune cells that phagocyte and oxide fat [53].

There is on-going research showing promising findings that adipocytes are a ready body source of cells that could be used for tissue-engineering reconstruction [54]. For instance adipose stem cells (ASCs) are a great promise for regenerative medicine applications. The use of de-cellularized human adipose tissue ECM combined with ASCs is a strategy that can be employed in the tissue engineering area [55]. Kim and collaborators designed a free-cell scaffold for adipose tissue regeneration; the aim was creating a specific scaffold to recruit cells into a desire cell type [56].Hence, research on adipocyte biomechanics has potential for evidence that could be applied to the development of methods for tissue construct. Indeed, a high proportion of reconstructive procedures involve repairing adipose tissue.

This case of adipocyte behaviour in the face of overload of fat illustrates how cells of the body are highly specialized systems that display impressive responses against mechanical forces outside and inside them. Thereby, any engineered treatment related to biomaterials for cells and tissues should rely on proper understanding of cell behaviour under unfavourable stimuli. In particular, biomaterials characteristics should aim to act in synergy with the natural cell systems in order to improve the conditions in which healing of cells and tissues can occur.

7. Future directions

Since tissue engineering appeared in the 1990s, research on biomaterials has increased and advanced greatly. Now these materials have specific characteristics depending on the tissue in which they want to be applied. Moreover, the physical characteristics (i.e. mechanical) of living systems are important in order to create artificial scaffolds. It is possible to reprogram cells through mechanical cues and synthetic constructs. However, the challenges consist of controlling such properties according to certain outcomes in cell behaviour. Also, the integration of more than one mechanical characteristic (i.e. external dynamic stimuli and matrix stiffness) imitating the *in vivo* conditions is required. Finally, further studies of mechanisms that direct cells to create new tissues are important to understand the way cells behave and respond to external and internal mechanical forces.

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