Review

The physics of tissue formation with mesenchymal stem cells

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Cells react to various forms of physical phenomena that promote and maintain the formation of tissues. The best example of this are cells of musculoskeletal origin, such as mesenchymal stem cells (MSCs), which consistently proliferate or differentiate under cues from hydrostatic pressure, diffusive mass transport, shear stress, surface chemistry, mechanotransduction, and molecular kinetics. To date, no other cell type shows greater receptiveness to macroscopic and microscopic cues, highlighting the acute sensitivity of MSCs and the importance of physical principles in tissue homeostasis. In this review, we describe the literature that has shown how physical phenomena govern MSCs biology and provide insight into the mechanisms and strategies that can spur new biotechnological applications with tissue biology.

MSCs respond to physical forces

Developing and adult tissues display an exquisite combination of physical, chemical, and biological processes [1]. Clinicians have successfully regenerated, replaced, or induced [2,3] tissue formation with limited knowledge of the processes that make functional tissue possible. To develop more successful treatments for regenerating complex tissues and treating diseases, known and novel molecular mechanisms governing tissue formation and maintenance must be investigated in the context of tissue physics. Musculoskeletal tissues and their cells have inspired some of the clearest examples of how physical phenomena can induce cells to form and maintain a tissue [4,5]. Because of their potential as cell sources in treatments of musculoskeletal tissue disease and trauma, MSCs have been the focus of many studies that aim to understand their biology and harness their therapeutic potential in a controlled manner [6]. To exploit this potential, the physical phenomena that underlie MSC behavior must be elucidated, controlled, and combined to promote useful biomedical applications.

In this review, we discuss the physical forces that stimulate MSC biology in the direction of tissue formation and maintenance, with an outlook on how these forces support the regeneration of musculoskeletal tissues. We focus on MSCs because of their sensitivity to many physical forces, as determined by the use of techniques [7] such as nanotechnology platforms and mathematical modeling. Through converging technologies, it has been possible to quantitatively elucidate MSC responses to physical forces from the macroscopic to the molecular level, where at different levels of organization in tissue, fluid statics, fluid dynamics, and surface physics act on MSCs through similar qualitative mechanotransductive and kinetic mechanisms (see Glossary). We describe all the physical phenomena reported to affect MSCs biology for three reasons: (i) to inform the reader thoroughly about the various stimuli without bias towards one phenomena in particular; (ii) to analyze systematically the literature according to the various levels of organization; (iii) to inspire new insights into MSC biology theory and to stimulate the development of novel approaches to control and implement stem cells in modern medicine. Intentionally, we do not describe all possible and published molecular mechanisms. We focus instead on the physical forces and how these may be transduced from the tissue level to an individual MSC because we want to highlight the key role that physics plays in controlling stem cell fate.

MSCs in static fluids

Static fluids continuously expose MSCs to two types of physical phenomenon: hydrostatic pressure (HP) and diffusive mass transport (Box 1).

Glossary

Adipogenesis: the process of making new adipose (fat) tissue by cells called adipocytes, which can arise from MSCs.
Bone marrow: the mass of tissue found in the interior of bones.
Chondrogenesis: the process of creating new cartilage material by cells called chondrocytes, which can derive from MSCs.
Extracellular matrix (ECM): an extracellular connective tissue that provides structural support to cells, among other important biological functions.
Fluid dynamics: the study of fluids in motion by convection, such as in bioreactors mimicking the vascular system.
Fluid statics: the study of fluids at rest, which defines the physical state of liquid culture medium in state-of-the-art MSC research.
Mechanotransduction: the activation of signal transduction pathways by mechanical (physical) stimuli.
Osteogenesis: the process of forming new bone material by cells called osteoblasts, which can originate from MSCs.
Shear stress: the stress (T) caused by a force in a direction parallel or tangential to a surface T=F/A, where F is the applied force, and A is the area of contact with F.
Surface physics: the study of the physical changes that alter the biochemical interactions at interfaces, thereby changing surface chemistry.
Trabeculae: a dense region of tissue, which in bone is porous and brittle.
Ultrasound: a cyclic pressure induced by sound waves at high frequencies (above 20 KHz).

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Box 1. Physical concepts relevant to MSCs

Hydrostatic pressure

HP refers to the pressure change in a fluid caused by an external force in a closed geometry (Figure 1, main text). HP is expressed as $HP=\rho gh$, where g is gravity, ρ is density, and h is height, assuming the fluid is incompressible. The external force (ΔP_{ex}), which can be that exerted by compression, tension, or ultrasound (Figure 1a, main text), increases HP, as $HP=\Delta P_{ex}+\rho gh$. The term 'ρgh' is negligible, because it corresponds to an increase in HP of approximately 100 Pa per cm of height. Thus, external stimulation is the main cause of pressure changes.

Diffusive mass transport

Diffusive mass transport occurs when the concentration of a molecule varies at different locations, creating a chemical potential. Diffusive mass transport is important throughout all musculoskeletal tissues, particularly in the regions away from blood vessels. In such cases, the chemical potential becomes a driving force for mass transport from regions of higher to regions of lower molecular concentrations. According to Fick's first law, the mass transport can be defined by a flow rate (j) of a molecule equal to $j=\rho Ddw/dy$, where ρ is the density of the tissue or medium, D is the diffusivity of the molecule in that tissue or medium, and the term 'dw/dy' represents the mass fraction (w) change of a molecule in a distance (y). The term 'dw/dy' is the driving force of j and the mathematical interpretation of concentration gradients.

Kinetics

Kinetics refers to the movement of molecules across the cell membrane, which is directly linked to the law of conservation of mass. This means that, in a closed volume, any molecule that enters will either stay in, go out, or be converted into some other molecule. In kinetic studies, any molecule (m) whose molar concentration changes in time (dC_m/dt) is defined by: $dC_m/dt = Cin_m - Cout_m + Rx_A - Rx_B$, where Cin_m and $Cout_m$ describe the molar concentration of m going into and out of the volume, respectively. Rx_A and Rx_B describe the conversion of molecules (Figure I) via spontaneous degradation or production by MSCs. In this manner, m could refer to consumed molecules, such as nutrients, or produced molecules, such as trophic factors or ECM components. Finally, conversion of molecules can occur through spontaneous reactions (Rx_A) that must be identified to make accurate estimates and predictions.

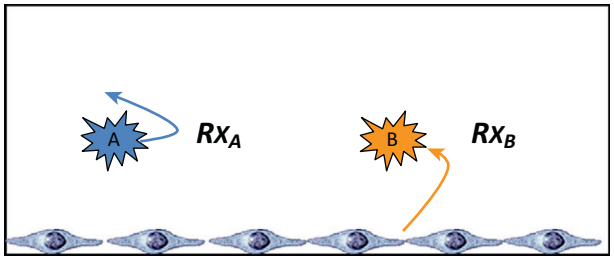

Figure I. Mass balance of molecule m in a volume of media contained in tissue culture plastic, where cells are located on the surface. Cin_m and $Cout_m$ are equal to zero because m does not enter or leave the container. Thus, concentration changes of m are only caused by reaction rates Rx_A and Rx_B, which depict spontaneous degradation (Rx_A) of molecule m and production of molecule m (Rx_B) by mesenchymal stem cells (MSCs).

Hydrostatic pressure

Studies that mimicked HP [4] showed that loadings from 1 to 50 KPa have an osteogenic effect, whereas physiological loadings from 0.1 to 10 MPa have a chondrogenic effect on MSCs (Table 1). Although the HP ranges are broad in most studies, probably due to the variability of measurements from different species and anatomical locations, studies suggest that the molecular mechanisms of bone remodeling [8] are similar in all mammals, but the amount of HP that triggers a mechanotransductive effect varies across species. For chondrogenesis of MSCs, although HP >0.1 MPa induces proteoglycans expression, HP >1 MPa [4] consistently stimulates the expression of a broader range of healthy cartilage markers [9], such as collagen II, aggrecan, proteoglycans, and sex-determining region Y box 9 (Sox-9). Yet, the introduction of factors such as transforming growth factors (TGF-β1 or TGF-β3) remains a more effective strategy to stimulate the chondrogenesis of MSCs [10] than HP alone (Table 1). Although these studies are performed using physiological levels of mechanical loading but supra-physiological levels of TGF (e.g., 10 ng/ml), the influence of both dynamic loading and HP on chondrogenesis of MSCs is maximized at lower concentrations of TGF [11,12].

Ultrasound also induces changes in HP (Figure 1a). Pressure applied by ultrasound increases the yield of MSCs from the human umbilical cord [13] and induces their osteogenic [14] and chondrogenic [15] differentiation. Typically, low intensities (25–35 mW/cm^2) induce osteogenic differentiation, whereas high intensities (30–200 mW/cm^2) induce chondrogenic differentiation (Table 1). It must be noted that the intensity threshold for osteogenic and chondrogenic differentiation is not yet clearly defined because ultrasound also depends on the frequency, which varies in the induction of osteogenesis with respect to chondrogenesis (Table 1). Although the volume of the sample and frequency of the ultrasound need to be considered for a complete pressure–ultrasound relation, it seems that these intensities can induce MSC chondrogenic differentiation in the same manner as loadings between 0.1 and 10 MPa. HP changes by compression or tension require physical contact with the stimulated body, whereas ultrasound does not (Figure 1a). Thus, sound waves may induce HP more homogeneously than might compression or tension, enhancing MSC viability and preventing MSC death.

The generation of HP is preceded by axial load, which means that there are numerous situations in the body with open geometry and MSCs exposed to axial load. Theoretically, axial and hydrostatic load could be considered complementary forces acting on MSCs. However, we suggest that axial load is negligible in some situations, because HP in the human body increases up to 18 MPa, such as in healthy human joints [16,17]. However, whether axial load precedes or follows HP through the extracellular matrix (ECM) to induce MSC differentiation depends on at least three factors, as a force transfers from the macroscopic to the molecular level (Figure 1). First, the geometry of a construct determines the distribution of forces and the amount of pressure that each MSC experiences, hence the type of differentiation. For example, forces applied on meniscus-like structures yield cartilage [18], whereas those applied on elongated structures result in either tendon substitutes [19] or cell death [20]. Second, forces

Table 1. Qualitative and quantitative aspects of physical phenomena on MSCs

Phenomena	Parameter	Effect	Refs
Hydrostatic pressure			
Compression (Human hips)	1.6–9.7 KPa	Osteogenesis	[4]
Compression (Sheep tibia)	15–50 KPa	Osteogenesis	[4]
Compression (Rat's femur)	2–8 KPa	Osteogenesis	[4]
Oscillatory Compression	1 MPa and above with TGF-β3	Chondrogenesis	[4]
Ultrasound	25–35 mW/cm^2, F=1.5–40 MHz	Osteogenesis	[14]
	30–200 mW/cm^2, F=1–1.5 MHz	Chondrogenesis	[15]
Shear stress			
Perfusion bioreactor	1×10^{-5} Pa	Osteogenesis	[40]
	1×10^{-4} Pa	Proliferation	[40]
	5×10^{-3} Pa	Mineralization	[41]
	0.1–1 Pa	MAPK signaling	[45]
Proliferation kinetics			
ECM (Dkk-1)	0.7 to 2 day^{-1} dependent on O_2 availability	Production	[71]
Alkaline phosphatase	1 to 3×10^{-6} mmol/min/cell dependent on shear stress	Production	[40]
	2×10^{-10} mmol/min/cell in static conditions	Production	[74]
Calcium	2 to 8×10^{-4} μg calcium/cell dependent on shear stress	Production	[40]
Oxygen	2.6×10^{-3} s^{-1} in static culture	Consumption	[70]
Glucose	0.2 to 0.8×10^{-12} moles/cell/h dependent on conditions	Consumption	[33,75,76]
Lactate	0.5 to 1.8×10^{-12} moles/cell/h dependent on conditions	Production	[33,75,76]
Amino acids	Values are specific for each amino acid	Consumption and production	[76]

are transmitted to MSCs according to the viscosity of the ECM of tissues with specific differentiation effects [21–23], as implied from the tuning of the mechanical properties of gels [21,22] and collagen fibers [23] to imitate the ECM. Third, forces induce changes of MSC morphology [24], which depend on the viscoelastic properties of individual MSCs [25]: elastic modulus of approximately 1 KPa, apparent viscosity of approximately 1.3 KPa/sec, and a height/width ratio of approximately 0.5. Although bone marrow cavities are protected against external loads by a mineralized matrix, where the pressure in all bone cavities depends on blood pressure [4], there is one important unifying characteristic to consider: how pressure transfers in a solid differs from how it would transfer in a liquid because of the pressure gradients that can be formed [26], thereby applying axial loads to the bone and changes in pressure to the cavities of the bone marrow. Thus, two situations arise: in a liquid (less viscous) environment, compression induces localized deformation, where only some cells in a construct sense the HP changes (Figure 1b), but may not sense axial loads. In a solid (more viscous) environment, all cells in the geometry sense both changes in HP and axial loads adjusting to them (Figure 1c). Consequently, how HP distributes inside a geometry may largely depend on the internal stiffness of cell–cell interactions with extracellular components.

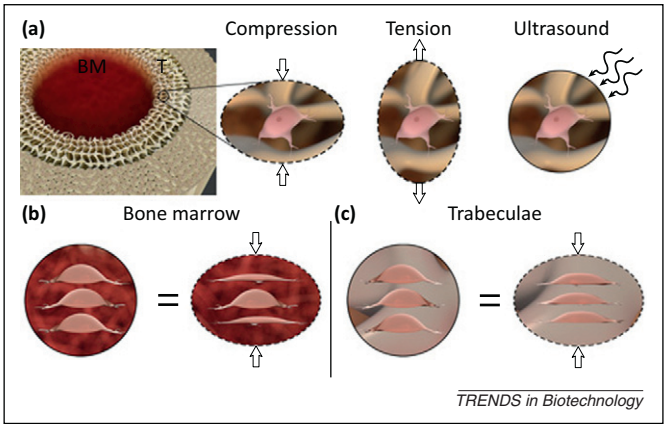

Figure 1. The hydrostatic pressure in bone is changed by external forces, such as compression, tension, or ultrasound, and is sensed directly by mesenchymal stem cells (MSCs) depending on the viscosity of the environment. Structural representation of the bone marrow (BM) and surrounding trabeculae (T) with a magnified spherical volume of trabeculae containing one MSC depicted under: compression, tension and ultrasound **(a)**. **(b)** shows an MSC in the less viscous environment of the bone marrow as opposed to an MSC in the more viscous environment of the trabeculae **(c)**, both under compression. Note that only some MSCs sense the volumetric compression of a spherical volume with a lower viscosity (e.g., bone marrow), whereas all MSCs sense the volumetric compression of a spherical volume with a higher viscosity (e.g., trabeculae).

Elucidating the HP distribution in complex 3D structures will further unravel the geometrical function of musculoskeletal tissues *in vivo*. In particular, how MSCs and ECM molecularly react to HP changes will be essential. Significant steps in this direction may be first taken using micro-fluidic devices, where HP can be controlled [27] and various components and viscosities of ECM can be defined according to their mechanotransduction potential.

Diffusive mass transport

Musculoskeletal tissues evolve blood vessels at defined distances to assist diffusive mass transport in maintaining viable MSCs. Thus, these tissues are highly sensitive to concentrations of various compounds, particularly oxygen [28], which stimulates differentiation [29,30], homing [31], angiogenesis [32], or death [33]. In MSCs, hypoxia at oxygen concentrations below 2% is known to promote: (i) proliferation, shown through increasing numbers of colony-forming units and the expression of cell cycle stimulator cyclin B1; (ii) stemness, inferred from expression of the markers octamer-binding transcription factor 4 (Oct-4) and Rex-1; (iii) protein synthesis, concluded from fibronectin production; (iv) osteogenesis, inferred from expression of alkaline phosphatase and osteonectin; (v) adipogenesis,

represented by expression of lipase; and (vi) chondrogenesis, shown by expression of cartilage markers. Hyperbaric oxygen (100% O_2) [30] can stimulate nerve regeneration by preventing inflammatory responses [34]. Furthermore, proapoptotic and proinflammatory signals [31] increase the adhesiveness of MSCs to endothelial cells. Fetal bovine serum concentrations ranging from 0% to 30% [32] stimulate the production of vascular endothelial growth factor and angiogenin. Lactate (above 16 mM) and ammonia (above 2 mM) [33] inhibit proliferation and cause MSC death.

Molecular concentrations are particularly important for MSC maintenance in 3D, which involves the essential nutrients that cells need to survive. Thus, diffusive mass transport must always be present to ensure that a molecular concentration in space (dw/dy) is never equal to zero for molecules that are necessary for cell survival. However, the profiles or steepness of these gradients are variable and depend on the diffusion constant (D) and kinetics of MSCs for a particular molecule. Therefore, concentration-gradient profiles must first be predicted [35] and then considered if one is to understand MSC biology, as is the case for trophic factors [36], where diffusive mass transport can help unravel the biophysical nature of transport of molecules in the formation and maintenance of malignant [37] and healthy tissues.

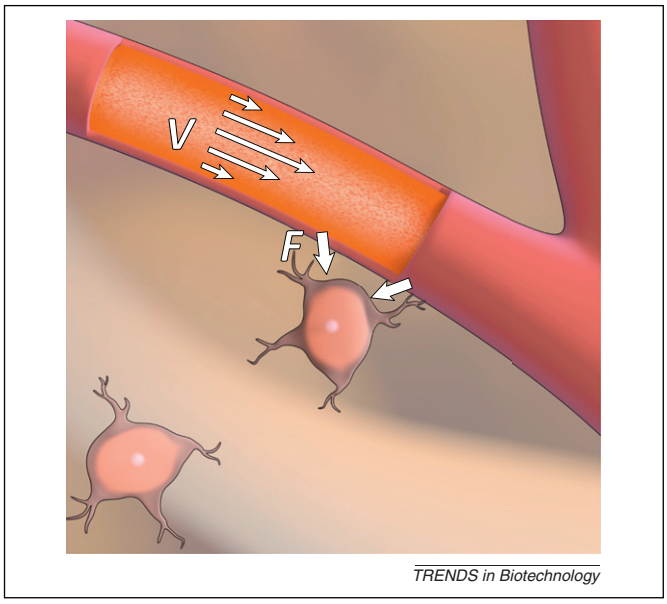

Figure 2. Mesenchymal stem cells (MSCs) respond to shear stress depending on their position with respect to the vascular system or other channels where fluid flows. Thus, the closer an MSC is located to fluid flow, the higher amount of force (F) that will be acting on it. As an example, shear stress is depicted in a blood vessel under laminar flow with fluid velocity (V) and molecular concentrations changing radially across the cylindrical vessel.

Fluid dynamics

Convection not only improves the supply of molecules to MSCs, but also causes shear stress, which can be stimulating or detrimental to MSC behavior.

Shear stress

The force acting on cells is caused by fluid flow in vessels or other channels directly in contact or close to an MSC (Figure 2) in tissues such as bone, and is forced through soft tissues, such as cartilage, during loading. Flow stimulates MSC proliferation and differentiation [38] with oscillatory flow increasing the MSC proliferation rate [39]. However, as shown in Table 1, increasing shear stress promotes osteogenesis [40] and mineralization [41], with shear stress orienting the organization of tissue [38]. For example, bone rods can be formed perpendicular to a surface under turbulent fluid dynamics with eddies of 250 μm [41]. In addition, shear stress (5×10^{-3} Pa) can also form bone rods parallel to the fluid flow direction in perfusion bioreactors [41]. Thus, shear stress also has structural implications in tissue formation with MSCs. The geometry and structure of matrices, as well as the fluid flow rate [42], determine the flow streamlines in different bioreactor configurations. Thus, bone rods are determined by the response of MSCs to eddies and flow. To control shear stress, different configurations have been designed to maintain a homogenous environment and a consistent effect on MSCs [40,43,44]. As a result, genes significantly expressed in response to shear stress (0.1–1 Pa) and its duration (10 min, 1 h, and 24 h) were identified [45]. In addition, combined shear stress and cyclic flexure have also dramatically accelerated tissue formation with MSCs within engineered heart valves [46].

The shear stress effect on MSCs to engineer cartilage, bone, and ligament [47] works in different ways. For articular cartilage, laminar flow patterns are better than either static (no flow) or turbulent steady flow. For bone, flow induces changes in the geometry, distribution, and orientation of bone-like trabeculae. For ligament, combining dynamic stretch and torsion improves cell differentiation, alignment, and functional assembly. The response of MSCs to shear stress shows that replacing the vascular system *in vitro* helps the formation of musculoskeletal tissues, but cannot replace the maintenance and control role of the vascular system. The role of shear stress in MSC

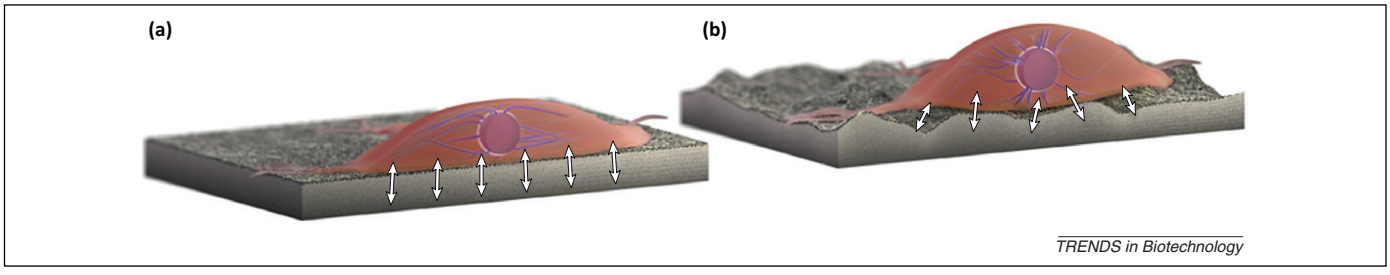

Figure 3. Substrate nanotextures interact with mesenchymal stem cell (MSC) membrane proteins to differentiate MSCs with high specificity depending on the flatness **(a)** or roughness **(b)** of a surface. The surface affects the cell shape through membrane proteins (↔) that cause variation in the force balance of the cytoskeleton (purple fiber network) in contact with the nucleus (red) inside cells, resulting in MSC differentiation.

biology has laid a strong foundation for the use of microfluidic systems [48] combined with mathematical modeling [49] to address questions, under fluid dynamics, about the effect on MSCs of gradients, cell communication, and ECM development.

Surface physics

Given that MSCs in culture require an attachment surface, the need for substrates has produced a broad range of materials that can influence MSC proliferation and differentiation. Through the use of methods that modify the nano- and microstructure of materials [50,51], the shape, proliferation, and differentiation of MSCs can be tuned by surface physics and chemistry (Figure 3), which interact directly with membrane proteins.

Surface chemistry

Due to the nature of the methods used to change surfaces physically, surface modification affects the number and type of bonds [50], thus intertwining the fields of surface chemistry and physics. A comparison of nano- and micro-sized surface modifications of the same material showed that MSCs cultured on nano-textured surfaces displayed a higher osteogenic differentiation than on micro-textured surfaces [52]. This suggested that it is necessary to explore MSC proteins, such as focal adhesion and membrane protein complexes, to explore the effect of surface modifications on MSC differentiation. Solely by modifying surface chemistry in a controlled manner, for example, the supramolecular organization of fibronectin can be varied without affecting protein quantities, making it possible to understand the protein interactions by which focal adhesions regulate MSC differentiation [53].

For the past 150 years, chemists and physicists have dealt with the nature of how different elements of the periodic table interact with each other to change and maintain protein conformation through bonds. Altering bonds and elements of a surface have maintained or promoted MSC proliferation and differentiation [51]. Cytoskeleton rearrangements during differentiation suggest that focal adhesions play a major role in this process of lineage commitment [54–56]. How the signal is transduced from surface modifications to the gene expression level to establish the lineage commitment can be deduced with the structural components of this process, which include at least focal adhesions, the proteins Ras homolog gene family, member A (RhoA) and Rho-associated protein kinase (ROCK), the actin–myosin cytoskeleton, mitogen-activated protein (MAP) kinase, and Wnt signaling-associated transcripts [54–56]. It is possible that quantities of a component might determine one lineage over another. For example, the number of focal adhesions induced by a surface could determine the differentiation lineage of MSCs.

Disorder

Musculoskeletal tissues are organized 3D structures, where the cellular organization is characteristic of each tissue. A fundamental aspect of this organization has been discovered through surface physics by showing that the degree of order on surfaces [57] can provide the optimal conditions for stimulating MSCs to form bone cells [58]. The implication of this is that there are degrees of order and disorder achieved by nature, which support tissue functioning, and some that do not. Disorder and tissue organization can inspire new stimuli for MSC differentiation. The degree of disorder translates into distances between cells that determine the structure of the ECM and affect transport phenomena and cell communication. The study of physical forces at the molecular level in MSCs has produced important advances in two areas: mechanotransduction and molecular kinetics, which are affected by surface physics, fluid statics, or dynamics to induce changes in MSCs.

Mechanotransduction

Mechanotransduction has been studied in MSCs in response to multiple biophysical cues. Physical forces induce a cascade of molecular, biochemical, and physical changes on membrane proteins, which tip the intracellular force balance displayed by cytoskeleton organization through contact with the nucleus.

Each MSC is basically a 3D structure. Determining its linear dimensions, such as height, can help determine how forces inside the cell (Figure 3) cause it to live, die, or differentiate. For example, a disc-like shape of MSCs has been correlated with maintaining a healthy intervertebral disc [59]. To help understand how MSCs control their shape, structural components in the cytoskeleton have been determined [55,56,60]. The cytoskeleton dynamics alone change so dramatically that, during osteogenic differentiation, the average Young's modulus of an MSC (3.2 kPa) transforms into that of mature bone cells (1.7 kPa) [60]. At the same time as the cytoskeleton mechanically adjusts, lineage-specific metabolite production [61] takes place, suggesting that these biomarkers are predictive of, and enrich, differentiation. Upon cytoskeleton disruption, such as by an electrical field, forces inside MSCs change and determine MSC protein biochemistry [62]. Elucidating the correct type and quantity of cytoskeletal components is essential to control MSC fate, as highlighted by the fact that MSCs can buffer 3–10 pN tension fluctuations [63], suggesting that the membrane and cytoskeleton are structurally unique and adaptable to external stimuli.

Mechanotransduction is establishing the molecular components necessary to maintain robustly tissue homeostasis. In addition, new theories on cell mechanosensing are opening new possibilities to control and induce cell and tissue organization [64]. Thus, it is becoming evident that mechanotransduction theory needs to be tested at the 3D tissue level, where MSCs are cultured under the presence of controlled fluid statics or dynamics. For example, the combination of theoretical tension fields [65] with experimentally measured forces [66] can unravel the rigidity of 3D [67,68] environments inducing MSC differentiation. This rigidity can be tested against assumptions made in tensegrity [64] to define force balances for cells and ECM within tissues. In this manner, it is possible to determine the contractility and molecular components necessary to understand musculoskeletal tissue biology and physics [69].

Proliferation kinetics

Kinetics has been used to describe MSC proliferation. This has produced several studies regarding the kinetics of MSC proliferation on tissue culture plastic and bioreactors [40,70–72]. So far, studies have dealt with the kinetic effect on MSCs of cell numbers [73], the 3D microenvironment [70–72], and shear stress [40]. The main contribution of kinetics to MSC biology has been to quantify proliferation and describe it in parameters that can lay the foundation to control, understand, and optimize research and biomedical uses of MSCs. Thus, MSC proliferation and differentiation have been characterized according to proliferation rates or growth rates and the production or consumption of essential molecules (Table 1), such as ECM [71], alkaline phosphatase [40,74], calcium [40], oxygen [70], glucose, lactate, ammonia, and amino acids [33,75,76]. This approach to control cell cultures has been successfully performed with vaccine and food production after the identification of major pathways and components involved in a biological process. In the case of MSCs, the list of components required for any biological process is arguably larger because MSC proliferation and differentiation are inherently more complex. However, systematic analysis of small sets of reactions can develop into quantitative descriptions of pathways and processes, such as trophism [36], where kinetics can elucidate the molecular quantities and conditions that trigger MSC homing, migration, and differentiation.

Concluding remarks

The stimulation of MSCs through physical forces holds numerous possibilities for fundamental and applied research. For 3D tissue culture, MSC molecular kinetics suggests that MSCs would be more therapeutically effective if they were concentrated in strategic locations in 3D scaffolds instead of homogeneously seeded throughout scaffolds. This could benefit tissue regeneration in three ways by: (i) reducing the number of MSCs and so the need for expansion; (ii) stimulating extensive implanted cell–host cell contact in desired regions around the scaffold; and (iii) preventing the exposure of MSCs to nutrient limitation *in vivo* after *in vitro* culture. For therapeutic effects to occur in realistic time spans with this approach, the diffusive mass transport and kinetics of biological factors are required. To cope with the technical limitations of tracking molecules in solution, imaging techniques [77–81] can contribute to monitoring molecules, such as trophic factors.

Furthermore, in a bottom-up approach to regenerate tissue with MSCs, each phase of tissue formation could be established according to the size scale of physical phenomena. First, the surface physics are defined for a specific MSC lineage. Then, fluid statics and dynamics that support the 3D organization of MSCs in a lineage are introduced. Physical phenomena combined in timed stages of regeneration can provide the most stable and efficient conditions for stimulating the robust creation of a tissue. In this regard, mechanotransduction and molecular kinetics provide the fundamental knowledge required to trigger mechanosensitive pathways in MSCs [65,82], where for example, as osteogenesis is initiated, the pre-vascularization and angiogenesis of bone [4] could be initiated through controlled shear stress in micro- and macro-bioreactors. Several studies discussed in this review show that physical phenomena change the shape and organization of MSCs, resulting in functional differentiated MSCs, where shear stress regimes could define the conditions for vascularization of mm-size bone constructs in bioreactors. Critically for bone tissue engineering, this means integrating shear stress data with biological cues to harness the vascularization properties of MSCs.

Many studies have indicated that physical phenomena significantly determine the biological response of MSCs *in vitro* [4]. On-line monitoring [83] of tissue biology in animal experiments [84] could provide some of the fundamental principles necessary to recreate natural tissue in 3D *in vitro* models, which is becoming a standard to test MSC (and other cell) responses, and lay the foundation for the development and application of MSCs in medical treatments [85,86]. To be able to find optimal conditions, high-throughput screening (HTS) has contributed to determining fundamental aspects of tissue biology [87–89], such as the effects of drugs and other molecules on diseased and healthy cells. The disadvantage here is that *in vitro* HTS screening can be biased with respect to the true conditions that regenerate tissue *in vivo* by, for example, producing false positives. Ideally, an *in vivo* screening system could be used to find optimal conditions for tissue formation. This would create a top-down approach to regenerate tissue, where biomaterials could be processed to create controlled environments for screening multiple conditions in animal models.

Acknowledgments

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