 doi:10.20944/preprints201901.0115.v1

Review Article

The Complicated Body in Motion: Rediscovering the Mechanical Stimulations in Cell Models of Human Disease

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Abstract

Thought runs through the mind like blood runs through our body to keep us alive. Like the mind, the body does not stay inert and is in constant motion. Not a single cell in our body is left inert unless cell is under stress or dying. These scenarios are reflected upon when a person is sick, the person lies in bed with less movement; however, is active when the person is healthy. The topic of mechanical stimulation has emerged due to the increasing understanding of the physical stimulations we face each day. Further understanding of the mechanically-regulated mechanism can help us explore the pathological events in a disease. Here, we reviewed the role of sensory proteins in pathological events that are observed in cardiomyopathy, cancer, respiratory, renal, obesity, genetics, physical injury and bacterial infection. Taken together, sensory proteins are mechanically-activated which assist reception of external physical stimulation and convert into biochemical to trigger intracellular signaling cascade.

Keywords: Motion; Inert; Mechanical stimulation; Sensory proteins.

 doi:10.20944/preprints201901.0115.v1

Introduction

The challenge of simulating mechanical stimulation as in vivo

In a healthy lifestyle, regular exercises are preferred over a sedentary lifestyle which may result to weight gain, weaken muscles, lower sense of well-being, and low self-esteem. Clinically, patients with difficulty in movement can be improved through exercising as in rehabilitation. In a vegetative state, individuals retain the desire to move despite being unable to move voluntarily. From a macroscopic view, biological entity requires movement, or physical stimulation in nature.

The challenge of simulating the dynamics as in vivo requires recapitulation of the external physical force and the cellular microenvironment. The cellular microenvironment represents a tiny unit of our entire body, receiving physical stimulations for normal function from the extracellular space. In this review, we summarized current research on the function of mechanical stimulations in human disease, and examined the types of mechanical stimulation for simulating the disease.

Sensory proteins

The physical force is sensed by the cells through a network of sensory proteins or mechanically-activated proteins such as G protein-coupled receptors (GPCRs) or ion channels which transmit the signal intracellularly[1] (Figure 1). Inside the cell, several types of proteins are activated, causing conformational change, protein-protein interaction[2]. In some cases, the intracellular signal is transduced into cell nucleus for transcriptional regulation. The major types of physical forces are compression, stretching, hydraulic pressure, and so forth. The physical forces are converted into electrical or biochemical signals.

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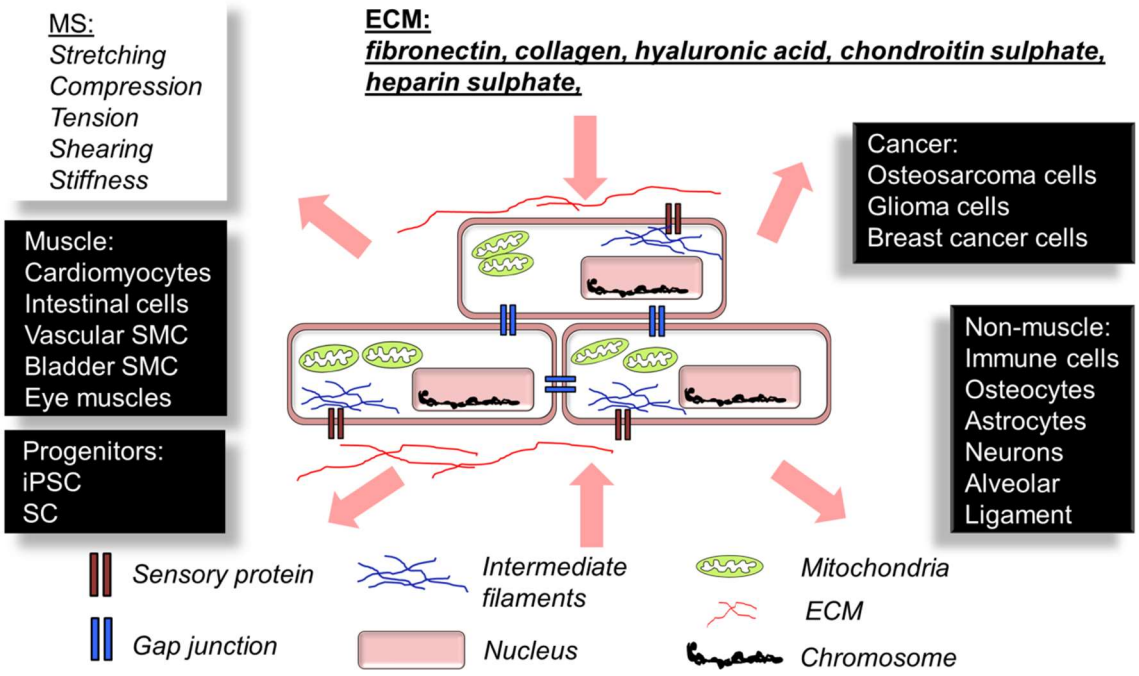

Figure 1. Illustration depicting the major components that sense mechanical stimulation in the in vivo microenvironment. The cells utilize sensory protein in the cell membrane to detect mechanical stimulations in the extracellular space which is filled with ECM. Through the sensory protein, mechanical signals are converted into biochemical signals through reorganization of cytoskeletal proteins which can transduce signal into cell nucleus to recruit transcriptional co-activators such as YAP/TAZ for gene regulation. The major types of cells that are known to experience MS in their microenvironment are: cancer cells, muscle, non-muscle cells, and progenitor cells. Abbreviations: MS: Mechanical stimulation; ECM: Extracellular matrix; SMC: Smooth muscle cells; iPSC: induced-pluripotent stem cells; SC: stem cells.

For instance, the physical stimulations are converted into electrical signals as our hearing and sense of touch[3]. The Drosophila sound-sensitive neurons express the ion channel NompC for transducing mechanical stimulation signal during hearing[4]. The physical presence of foods, odors, and sights are changed into biochemical signals as our tastes, smells, and vision, respectively[5]. The two types of proteins that receive or sense the physical stimulations are ion channels and G protein-coupled receptors (GPCRs)[6]. The proteins that sense these physical stimulations are called sensory proteins. For physical stimulations to be converted into either biochemical or electrical, the cells require expression of sensory genes. For example, the sensory gene rhodopsin is expressed in the retina cells of the eye, producing the GPCR that is conjugated to chromophore. Upon sensation of physical light stimulation, the GCPR is activated by light photons, initiating a biochemical cascade.

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Mechanical stimulations sensed by various types of cells

The cells of different organs differ from one another in that they have different functions (Figure 1). Nonetheless, different cells can sense the same physical stimulation. For example, the eyes see light through sensing light photons, the skin sense the light through detecting warmth caused by light photons. Another example is the ear senses sound through vibration waves, the hand senses sound through vibrating medium or object[7]. Inside our body, the cells sense the beating heart, proclamation of its living status. The change in the heart rate would be sensed by other cells in the body, sending out an alarm in case of a sudden increase in heart pressure. Apelin receptor (APJ), a kind of GPCR expressed in the heart, is associated with cardiac hypertrophy, and heart failure[8].

Apart from the heart which is the dynamic center of being alive, other major organs such as the lungs also receive and experience physical stimulation[9]. As we breathe, the alveolar cells in the lungs expand, and relax as we breathe out. Sometimes over-breathing due to exercise might cause shortness of breath due to insufficient lung capacity. In other words, the lungs cannot provide enough fresh air at the required amount of time. However, over-breathing might not cause any visible or detrimental health problems. In patients who are on ventilator for breathing support, the consequence is more prominent. Many previous studies have reported that patients` lungs are literally injured by the mechanically-controlled ventilator. Mechanical stretching plays an critical part in maintaining lung structure[10]; however, due to over-stretching of the lungs, the alveolar type 2 cells produce less amount of surfactant which could protect the lungs from inflammation and injury[9].

Apart from physical stimulations that seem to originate from the external surrounding, there are those that originate from within the organs such as the intestinal movement, uterus contraction, blood pressure within blood vessels, bladder pressure, liver, and so forth. The blood vessels provide the body warm temperature, as well as fresh air through oxygen carrier, the red blood cells. However, blood vessels are not just fresh air provider, but also carry nutrients and required stem cells and hormones throughout the body. The structures of blood vessels are found to express stretch-activated ion channels (SAC) known as Piezo. The Piezo is expressed on endothelial cell membrane which can sense the pressure exerted by blood pressure onto the walls.

Despite the fact that brain neurons seem to have no motion and do not receive physical stimulation as dynamic as the beating heart at any given time, the SAC Piezo1 and Piezo2 were first found in neuronal cells called N2A cells[11]. It is plausible to speculate that the neurons are not in dynamic but still require sensory proteins to sense their surroundings. The neurons detect mechanical stimulation by converting the

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stimulation into biochemical signaling, changing the organization of intermittent filament inside the cells[1-2]. The protein Piezo1 has been found to be expressed in human cardiomyocytes and responded to cyclic stretching[12]. In the digestive tract, movement is important for digesting foods. The contraction and relaxation of intestinal muscle cells is found to enhance differentiation of progenitor cells, and also promote food digestion through calcium ion level increase via Piezo[13].

Furthermore, the liver is a major organ that is relatively less motile compared to the beating heart. Despite the fact that liver is less dynamic in nature, it is covered with immense amount of blood vessels through the hepatic portal artery and vein. In normal liver, the stellate cells are quiescent, residing in G0 phase of cell cycle. However, previous study showed that mechanical stimulation such as stretching can activate the stellate cells, differentiating into myofibroblasts which lead to liver fibrosis[14]. Furthermore, the blood vessels carry blood, thereby it is pulsatile with certain amount of pressure pressing against the blood vessel walls. Therefore, the liver is filled with dynamic of pulsating blood vessels.

Mechanical properties in 2D and 3D cell model

The current challenge in creating cell model that closely resembles in vivo is finding the suitable 3D scaffold that sustains mechanical stimulations. Evidence showed that mechanical stimulation has various beneficial effects on the cells being stretched. The reason is because cells have mechanical properties in which they respond to maintain normal functions. In vivo, the 3D microenvironment is filled with several major types of extracellular matrices such as collagen, fibronectin, hyaluronic acid, chondroitin sulphate and heparin sulfate[15]. Stretching is found to enhance angiogenesis, cell proliferation, as well as stem cells homing. In dentistry, orthodontal force or stretching is used to pull teeth so that dental stem cells, periodontal stem cells at the root of the teeth can be activated[16]. The mechanically-activated stem cells can become bone cells which might involve GTP-binding protein such as Rho[17], filling in the empty spaces in the tooth cavity. In vitro studies have found that mechanical stretching can promote stem cells growth[18]. Furthermore, the expanded stem cells can have clinical applications because of larger in cell number.

The greatest challenge in assessing disease as complicated as cancer is the growth of cancerous tissue or tumor. In vivo, the tumor grows into a detectable size, becomes a scar that is difficult to be cured. With the detectable size, the tumor also has stiffness that is usually higher compared to normal tissue[19]. In vitro experiments have shown that change in stiffness can alter cell proliferation, even promote cancer growth. Adding to the mechanical property of cells, including the cancer cells, the tumor stiffness itself is

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promoting tumor growth[20]. Ideas have begun to emerge that tumor growth might be affected when the sensory proteins in the cancer cells were inhibited. As a result, the use of 3D scaffold combined with a mechanical stimulation has become a major focus in recent cancer study. Furthermore, cancer study by organoid culture requires a 3D shape, in short, a depth to the culture system. Since previous study showed that stiffness of tumor is correlated to metastatic grade, the depth in 3D is believed to better mimic in vivo microenvironment, so is the mechanical property of cells[21]. In 2D culture, the mechanical property of cells is manifested by cell tension through adhesion onto the culture surface, and often void of any mechanical stimulation. As mentioned before, the mechanical stimulations are converted into signals in the cells through sensory proteins. It is plausible to speculate that non-dynamic culture system is unable to exert the function of sensory proteins when compared to culture supplied with mechanical stimulation.

Mechanical properties of diseased microenvironment

To investigate the role of mechanical stimulation, the pathological events such as angiogenesis, inflammation, epithelial-to-mesenchymal transition (EMT), hypertrophy, hypertension, cell death, and so on have been focused (Table 1). Many of these pathological events are affected by mechanical stimulation, thereby understanding the role of mechanical stimulation in disease can help find a cure.

Table 1. Mechanical cues manifestation in various pathological events.

	Disease/ Injury	**Pathological events**	**Mechanisms related to mechanical stimulation**	**Ref.**
1	Cardiomyopathy	Hypertrophy	Cardiac hypertrophy requires APJ sensory protein	[28]
		Hypertension	1. Vascular architecture development requires SAC Piezo1 in the arterial wall 2. Remodeling of arterial wall due to increased blood pressure requires Piezo1 regulation	[22]
		Failure	Stretched-induced angiotensinogen (Ageon) is regulated by p38α MAPK through pJNK down-regulation	[29]
2	Cancer	Cell migration	The stiffness in cell-to-ECM interaction during cancer progression enhances cell migration through invadopodia	[19-20]
		Cell invasion	Breast cancer cell migration is enhanced by compressive force	[30]
		Angiogenesis	Angiogenesis is enhanced by cyclic stretching which also aligns the endothelial sprouts perpendicular to the stretching force	[31]

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	Disease/ Injury	Pathological events	Mechanisms related to mechanical stimulation	Ref.
		Fibrosis	Hepatic stellate cells undergo fibronectin fibril assembly through direct effects of thrombi and mechanical strain which drives hepatic fibrosis	32
		Muscle wasting	Lewis lung carcinoma cell conditioned medium decreases mTORC1, protein synthesis signaling in stretched myotubes	33
		EMT	Mechanical ventilation increases lung hydroxyproline and the mesenchymal markers α-SMA and Vimentin in mice	34
		ECM rigidity	MSC differentiation requires tension from ECM stiffness to upregulate YAP/TAZ	35
3	Respiratory	Bronchi	Mechanical stress resulted from lung inflation in human bronchi tissue led to leukotriene-E4 increase, an inflammatory factor	36
		Lung inflation	Piezo2 over-expression in sensory neuron causes apnoea in mice	37
4	Renal	Renal fibrosis	Stretching of renal tubular epithelial cells increases Pyk2 which is associated to renal fibrosis in mice	38
		Ureteral obstruction	Stretching of mouse proximal tubule and collecting duct cells cause apoptosis	39
5	Obesity	Fat tissue	Mechanical stimulation with a 5-hour refractory period decreases TNF-α gene expression and mitigates obesity-triggered fatty tissue dysfunction in mice	40
		Diabetes	Static, passive muscle stretching lowers blood glucose	41
6	Genetics	Aneuploidy	Neuroblast of Drosophila elongates or stretches to avoid aneuploidy through preventing cytokinesis failure	42
		Abnormal muscle development	Muscle development in *C. elegans* requires muscle-induced tension to sustain PAK-1 and PIX-1 expressions	43
7	Physical injury	Cell death	Stretch-induced traumatic brain injury model increases intracellular calcium and cell death	44
		Inflammation	Stretching for 10 minutes, twice every day reduces inflammation in rats	45
		ECM remodeling	Stress in ECM activates latent TGF-β1	46
		Delayed wound healing	Shear stress by fluid flow triggers TGF-β1 release causes delayed wound healing in human corneal epithelial cells	23a

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	Disease/ Injury	Pathological events	Mechanisms related to mechanical stimulation	Ref.
8	Bacterial infection	Mitochondrial fission	Mechanical stress caused by bacterial infection leads to mitochondrial fission	[47]

Abbreviations: APJ: Apelin receptor; mTORC1: mammalian target of rapamycin complex 1; YAP: yes-associated protein 1; TAZ: Tafazzin; Pyk2: Pyruvate kinase 2; Piezo: Piezo Type Mechanosensitive Ion Channel Component; MAPK: Mitogen-activated protein kinase; pJNK: phosphorylated c-Jun N-terminal kinase; EMT: Epithelial-to-mesenchymal transition; PAK-1: P21 (RAC1) Activated Kinase 1; PIX-1: PAK (p21-activated kinase) Interacting eXchange factor; TNF-α: Tumor necrotic factor-alpha.

Starting from the heart, the GPCR APJ senses the increased cardiac pressure which could enhance hypertrophy. However, when the APJ is knocked out, hypertrophy is inhibited[8a]. Supporting the role of sensory protein in cardiac disease is remodeling of blood vessel structure during hypertension through the Piezo1 protein[22]. Secondly, in cancer, mechanical stimulation might cause accumulation of collagen which promotes fibrosis and inflammation. The stretched collagen is found to activate TGF-β1, and is released from the collagen matrix to further stimulate cells[23]. The cells stimulated by TGF- β1 might promote further TGF- β1 release. Furthermore, higher level of exercising has been linked to reduced circulating cancer cells[24], thereby leading to decreased metastasized tumor growth. From the point of view of immune system, mechanical stimulation might affect the immune cells through activation of macrophage. It is speculated that activated macrophage might be associated with a stepped-up immune system, therefore, a stronger immune system. The hypothesis is that physical stimulation through exercising affects our health at molecular level.

In general, a healthy individual is encouraged to maintain certain level of exercise all the time. The scientific reason behind this is that exercising the cells can affect cell metabolism. In vitro, mechanical stimulation can disrupt function of fat cells. In vivo, mechanical stimulation leads to dysfunctional fat tissue in mice. At macroscopic level, lack of movement is associated with pressure ulcer development which is a form of inflammation. However, excessive exercise might cause negative effects on the cells. Instead of converting physical stimulation into intracellular signals, the sensory proteins might be overloaded with increased physical stimulation. The overloaded cells might undergo apoptosis or have the pain sensation through stimulated neurons. The signaling pathway of pain in neurons has been attributed to neurotransmitter through amyloid beta fiber in sensory neurons, or through myelinated afferents and activation of NK-1 receptor to the spinal cord where pain sensation is sent to the brain[25]. Here, mechanically-

 doi:10.20944/preprints201901.0115.v1

activated ion channels such as Piezo1, and non-mechanically-activated ion channels such as TRPV1 are expressed in the neurons. However, the role of these mechanically-activated ion channels in the pain sensation requires further investigation.

Our daily life is filled with surprises of mechanical stimulation that can be of accidental. The accidents may cause physical injuries to our skin, brain, or any organs. To heal the wound, scientists have investigated the use of tension to encourage cell regeneration through promoting mesenchymal stem cells homing[26]. In vitro study showed that stretching force modulates integrin to promote fibrosis during scar formation[27]. Therefore, it is important to convey the amount of mechanical force to minimize scar formation. Since it is known that physical stimulation affects cells at molecular level, rehabilitation can be further improved through modulating the cells at gene level. Thus, the emerging trend in rehabilitation in recent years is mechanotherapy.

Concluding remarks

In recent years, 3D with additional mechanical stimulation culture system has been the rising trend in basic research. With advancement of knowledge in mechanical stimulations, technology for simulating the natural physical forces can be performed in the laboratory. The types of natural forces for mechanical simulation can be categorized into five main groups: 1. Cyclic stretching; 2. Passive stretching; 3. Cyclic compression; 4. Passive compression; 5. Shearing by fluid. New study in the future regarding diseased microenvironment needs to incorporate these mechanical stimulations into their models. Scaffolds that are stretchable are increasingly important for development of cell model because of the structure and architecture, the depth that is not available in 2D culture system. In summary, mechanical stimulations play important role in biological systems.

Acknowledgements

We would like to give special thanks to TAIHOYA (Kaohsiung, Taiwan) for ideas in mechanical stimulation technology.

Author contributions

Conceptualization and writing of this manuscript were contributed by Tzyy-Yue Wong; Yu-Kai Tseng and Tung-Chen Yeh were supportive of the mechanical stimulation ideas from many aspects, including conceptualization; Rong-Chang Jhong, Yue-Fang Wang, and Hui-Yu Chang were responsible for references gathering and organization; Pei-Wen Cheng carried out review and editing work.

Funding

There is no funding for this manuscript preparation.

Conflict of interest

The authors in this study declared no competing interests.

 doi:10.20944/preprints201901.0115.v1

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