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The Mechanoadaptation Concept of Cells

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Abstract. Almost all cells in the human body are subjected to mechanical stresses. These forces can vary from a few Pascals (shear stress) to some mega Pascals (on hip cartilage). It is now well known that mechanical forces have a decisive effect on cellular physiology. In 1880, W. Roux introduced *the concept of functional adaptation; which* can be defined as a quantitative autoregulation controlled by stimuli like mechanical forces. These stresses influence functionality and cellular metabolism and can lead to appropriate tissue remodelling by triggering a cascade of reactions (mechanotransduction), being the signal for the adaptation of cells and tissues. However, although the main biological effects of mechanical forces are well documented, the relation between mechanical forces and physiological phenomena is largely unknown. In this paper, some effects of mechanical stresses on different cells (mesenchymal stem cells, bone cells, chondrocyte, endothelial cells, vascular or muscular cells, etc.) are summarized.

1. Introduction

A fundamental property of living tissues is their adaptation to the environment and to movement (a property described by Roux at the end of the 19th century) which is defined as a quantitative autoregulation controlled by functional stimuli. Roux wrote «*many cells are influenced by functional stimuli, the nerve, muscle, gland cells by the related electric pulse, bone cells and connective tissue cells by pressure and tension*».

Movements of the body are at the origin of local mechanical stresses and constitute stimuli that can lead to a modification of the cells biological behaviour [43, 82]. These forces range from a few Pascals on the vascular wall (shear stress) to several mega Pascals on the cartilage of the hip [2, 8] and can influence the cellular metabolism and lead to appropriate tissue remodelling [83].

Mechanical approaches (mechanobiology) now make it possible not only to better understand the observed tissue remodelling but also to develop *in vitro* tissue repair (tissue engineering) [78,84,92]. However, it is important to recall that while the exponential development of research and applications is recent, the first observations on mechanical tissue adaptation and structure/strain relation of tissues were realized many years ago [60,95]. In 1866, Meyer [60] suggested during a conference in Zurich that: «*the spongiasa showed a well-motivated architecture which is closely connected with the statics and mechanics of bone*». A mathematician, Culmann, [82] remarked that the shape of a cantilever beam in civil engineering was the same as the head of a femur and started to develop the first theoretical approach. Meyer then formulated his ideas in three questions: «*is it possible that structures like those observed are formed by static* (*force-equilibrium*) *conditions? What is the internal metamorphosis that makes structures so "fit to serve"? Can these structures be understood if one adds to the external loads the mechanical*

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influence of the traction of muscles and ligaments?». Three years later, a German surgeon, Wolff [94, 95], wrote «*every change in the shape and function of bone or of their functions alone is followed by certain definitive changes in their internal architecture and equally definitive secondary alterations in their external confirmation in accordance with mathematical laws*». He thus described the phenomenon of tissue remodelling (without defining it in the strictest terms).

To explain this mechanical adaptation, a four step mechanism proposed initially for bone, can be extended to most of the cells involved:

1. Mechanocoupling, involving the conversion of the applied forces or physical phenomena (i.e., cardiac pressure induces blood flow or interstitial fluid flow) into a signal acting on the cells.
2. Conversion of the various forces (primary or secondary) into electrical, chemical or biochemical responses.
3. Transduction (i.e., intracellular conversion of a signal into final signals).
4. Cellular responses: cell differentiation, up- or down-regulation of genes, cellular proliferation, matrix synthesis, release of autocrine or paracrine factors, etc.

The modifications caused by mechanical forces (pressure, shearing, elongation) are now considered to be determining factors in our understanding of many physiopathological mechanisms (osteoarthritis, inflammation, vascular diseases, thrombosis, atherosclerosis...) and also in the progress of tissue reconstruction [33, 34, 48, 79, 90–92]. Furthermore, the regeneration of certain tissues such as bone or cartilage is now possible by cell therapy combined with a biomaterial vector and/or support, a concept still called "tissue engineering". However, several questions remain to be answered, in particular:

- How do the mechanical stimuli intervene in the regulation of the cells in the area of damage?
- Does the repair tissue obtained acquire and/or preserve the physicochemical and biochemical properties of the native tissue when it is subjected to mechanical stresses?

Research in mechanobiology can be clinically applied in many fields such as in particular vascular, cardiac and osteoarticular and the most recent works show that the impact of the stresses is specific to the cells concerned and that the mechanical stresses are involved in different physiological processes: for example by the production of the extracellular matrix (i.e. cartilage, blood vessels), specific secretions (i.e. production of NO, prostaglandins by the endothelial cells) or for the induction by intercellular communication of specific functions, hence the interest shown by pharmacologists in the study of new molecules. Moreover, this new knowledge led to the development of tissue engineering, that is to say, the development of replacement tissue *in vitro*, with bioresorbable vs. non-bioresorbable supports and of cells "cultivated" in a physiological mechanical environment (e.g. bone, cartilage, vessels...). So the phenomenon of cellular transplantation for tissue repair particularly using stem cells opens the way for new therapeutic opportunities.

2. Mechanical Forces and MSCs

A general problem in the understanding of MSCs is their ability to differentiate in different tissues (bone, cartilage, cells of blood vessels...) and the link to tissue function and dysfunction. With the recent advances in genetic research, we start to understand the different mechanotransduction pathways of stem cells and the cellular mechanisms responsible for the differentiation of stem cells into specific cells of tissues [50]. This new research field can lead to future clinical therapies [5, 15, 30, 51, 69, 74].

During the past 20 years, many researches where conducted on the influence of mechanical forces on MSCs. The importance of shear stress was recognized as a regulator of cell functions. For stem cells we can underlined the work of Adamo *et al.* [1] who showed that fluid shear stress is important in biological properties of MSC. It was found that mechanical forces induce cellular differentiation through the activation of specific signalling pathways, but many points are still unclear today. We can also underlined different researches which showed that cyclic stretch induces the differentiation of bone marrow MSCs toward smooth muscle cells [63]. Other studies

demonstrated differentiation from stem cells after, cyclic stretches, shear stress or pressure. The direction and type of the force stimulation (pressure, shear, tension) plays a major role in the cells differentiation. As an example compression or shear alone is insufficient for chondrogenic differentiation of MSCs, but compression and shear induce increase in a cartilage specific gene expression.

Other researches focus on the role of mechanical stimuli in the cellular environment (surface topology, cell shape, matrix stiffness) on the phenotype of MSCs since cells are sensitive to their microenvironment acting as a signal generator, and respond by their shape adaptation. Engler *et al*. [30] showed the role of matrix stiffness varying from soft to relatively rigid on MSCs differentiation. They showed that when the cells are cultured on a rigid matrix (25 to 40 KPa) osteoblastic differentiation is observed but for intermediate stiffness (8 to 7 KPa) myogenic phenotype is obtained and for soft surface (0,1 to 1KPa) neuronal phenotype is observed. These observations show that the matrix mechanical properties is one of the important factors for the differentiation of MSCs into a specific lineage but the exact pathways by which the environment determines cells maturation is today unclear, although, as mentioned above surface topology of the matrix environment affects MSCs maturation.

3. Influence of Mechanical Forces on Bone and Bone Cells

3.1. Introduction

Bone is a dynamic structure. Its density changes with the activation of bone forming cells (osteocytes, osteoblasts) and resorbing cells (osteoclasts) driven by mechanosensor cells (osteocytes), and its porosity can change as a result of pathological conditions or as an adaptive response to mechanical or physiological stimuli [6, 14, 16–18, 23, 29]. The hardness of bone comes from its mineralized matrix composed of collagen fibers which gives to bone its tensional stiffness. The bone matrix is continually renewed by osteoblasts which are produced by stem cells. Osteoclasts are phagocytic cells formed in bone marrow which physiologically absorb bone at that the same rate of bone deposition in homeostasis conditions. Finally osteocytes, the third type of bone cells, are differentiated osteoblasts trapped in the bone matrix. Changes in mechanical loading are reflected in skeletal modifications as a result of increased loading in athletes and many investigators have advanced that mechanical loading is crucial to the development of skeleton [49, 64, 91]. The idea that bone adapts to its mechanical environment is not new. Scientists observed that cancellous bone structure was correlated with mechanical forces [19, 24, 31, 37, 38]. But the first theoretical approach referring to the relationship between the shape and structure of the femur was that of Meyer [60] and Wolf [94,95]. What is called Wolff's law incorporates different concepts (optimisations of strength with respect to weight, alignment of trabecular structure with stress directions and the self-organisation of bone structure after mechanical stimulation). Wolff [95] published different papers on the "law of bone transformation" by affirming "there is mathematical correspondence between cancellous bone of the head of the femur and the trajectory of Culmann's crane that the compact bone is nothing but a compressed cancellous bone and that bone will increase its density and strength in areas exposed to stress".

In 1987, Frost [32] proposed a model known as "mechanostat of bone adaptation" which attempted to maintain an optimum level of bone strain. Thus for weak strains (0.02%) there is tendency for resorption whereas average strains (0.05 to 0.1%) are considered to be physiological. Moreover, it was shown that, in vivo, bone formation is not induced by a static load but is simulated by a minimum periodic load (frequency $>$ 0.5 Hz) and that low frequency loading inhibits its formation, this observation can be explained by a reduction in the flow of extracellular fluid and blood. This hypothesis was verified by Turner et al. [88] who showed that the bone formation of the tibia in the rat subjected to a bending load of 64 N was 6 times higher than that of the contralateral control.

It is important to recall that bone is a dynamic structure and that its structure changes as a result of pathological conditions or as an adaptive response to mechanical or physiologic stimuli [39, 70, 73, 75, 76]. Changes in mechanical loading are reflected in skeletal modifications as a result of increased loading in athletes for example and many investigators have advanced the view that mechanical loading is crucial to the development of the skeleton [40]. Other scientists observed that cancellous bone structure was correlated with mechanical forces.

But as mentioned above, the first theoretical approach referring to the relationship between the shape and structure of the femur was the one of Meyer (1866) and Wolf (1869). The known Wolff's law [94, 95] incorporates different concepts (optimisations of strength with respect to weight, alignment of trabecular structure with stress directions and the self-regulation of bone structure by cells after mechanical stimulation). It is showed that, in vivo, bone tissue can undergo a relative strain of 0.3% without damage but beyond 2% a fracture will occur. Following these observations, many studies have been published on the influence of stresses on bone remodelling [6] based mainly on mechanical adaptation, also listing the mechanisms by which cells convert physical stimuli into metabolic responses [77, 96]. From all this, the four-step model cited in the introduction was proposed. Out of all these findings, we can assume that static load inhibits the differentiation of osteoblasts, decreases RNA and protein synthesis and stimulates the generation of osteoclasts, whereas a cyclic load leads to an increase in the formation of bone tissue and reduces bone resorption. In addition, a cyclic strain increases the synthesis of PGE as well as collagen, vinculin, calmodulin and vimentin synthesis. The same applies when osteoblasts are subjected to cyclic stretching.

In vivo the consequences of this adaptative relationship is showed by the activity – related increase of bone mass seen in athletes and the loss of bone tissue during immobilization or bed rest after fracture [40]. Still, despite of the clinical significance of the adaptative process, little is known on the occurring mechanisms.

3.2. Mechanical Forces and Osteoblasts

The mechanisms for osteoblasts differentiation and their interaction with osteocytes and osteoclasts are still not fully understood [41]. The cells sense their environment by the stimulation of mechanical load and fluid flow. Bone structure is maintained by osteogenesis, and resorption made by the osteocytes, which are the most abundant cells in the matrix, considered as the mechanoreceptor to induce osteogenesis. It was showed that fluid flow induces intracellular calcium mobilization, production of prostaglandin E2 and NO and increased genes expression for osteopontin and cyclooxygenase. In other respects mechanical stimulation induces secretion of growth factors (IGF, VEGF, PDGF, TGFβ, BMP ...). These factors which are non-specific to osteoblasts are considered as regulators of osteogenesis and activate transcription factors, such RunX2. It was also showed that mechanical forces induce secretion of humoral factors such as cytokines, growth factors and hormones.

Another parameter influencing bone osteogenesis is fluid flow driving electrolytes through the media and thus inducing streaming potential which can activate bone cells.

A constant compression load of osteoblasts induces production of inflammatory cytokines, and interleukin 1β (IL-1β) synthesis is increased in an autocrine fashion and has a role in the RANKL-RANK signalling pathway stimulating osteoclastogenesis. The survival behaviour of osteoblasts is mostly force-dependent and lack of gravitation leads to their apoptosis. In the same way, a lack of mechanical stimulation leads to osteoblasts apoptosis and osteoporosis. Osteoblasts govern osteogenesis through interactions with osteocytes. However, the role of mechanical stimulation is not well understood.

In summary, mechanical forces lead the remodelling of bone [45] *and appear to act via cellular strain to maintain the physiological balance. But the current occurring mechanism is incomplete and should be extended to the understanding of the expression of transcription factors or modifications in cell phenotype.*

4. Mechanical Forces, Chondrocytes and Articular Cartilage [80, 82, 89]

Articular cartilage, which is the bearing material lining the bone, is a complex tissue composed of only one cell type (chondrocyte) in a matrix whose properties vary according to the nature of its fibres and depending on the thickness. Mechanical injury to articular cartilage has considerable variation in effect depending on the nature of injury (superficial, deep...) and on the extent of the lesion [10, 12, 21, 47, 81]. Therefore in a partial-thickness defect, collagen fibrils are mostly perpendicular to the defect surface in the superficial zone, parallel in the middle zone and perpendicular again in the deep zone.

It has been known for almost 100 years that cartilage responds to mechanical stimuli and it has been shown that immobilisation decreases the capacities of chondrocyte synthesis [41,93]. Thus load-bearing surfaces in joints are thicker than nearby non-load bearing surface of the same joint with a higher proteoglycan concentration. It was observed that local lesions resulting in the appearance of osteoarthritis are often localised in zones subjected to strong strain, suggesting that an excess of mechanical stresses is one of the factors involved in this pathology. Because of its particular structure, it is helpful to consider cartilage as a biphasic system (water and collagen – proteoglycan matrix). Its mechanical properties are complex and change with the developed strain, in connection with the movements of fluids and related to the stresses applied, which is one of the main ways of supplying nutrients. The cartilage follows a complex nonlinear viscoelastic law and has a very low friction coefficient compared with all known artificial materials. This coefficient depends not only on the measurement conditions (static or dynamic) but also on the surrounding fluid. In particular, its elastic modulus varies with the rate of loading. At very slow loading rates, this modulus is only a few MPa, but at physiological rates of dynamic loading, it is around 500 MPa. One important factor that enables cartilage to resist deformation is its high osmotic pressure, which increases rapidly with an increase in the density of fixed negative charges, leading to a hyperosmotic and acidic environment in the pericellular space of the chondrocyte.

In vitro studies on chondrocytes in culture showed that mechanical stresses play a key role in the synthesis and degradation of the cartilaginous matrix [71]. Thus, a static load alone leads to a decrease in the synthesis of two major components of the matrix (proteoglycan and collagen), results in the loss of cartilage in the matrix and triggers proinflammatory effects. Static persistent compression leads to loss of water and consequently to an increase in fixed charge density (an increase in cation concentration, a decrease in anion concentration, and a hyperosmotic environment). In addition, compression causes chondrocyte deformation. Periodic strain induces the synthesis of numerous compounds of the matrix (e.g. stimulation of prostaglandin or GAG synthesis). The frequency and amplitude of the strain are important factors (frequencies from 0.1 to 1 Hz increase the incorporation of ^{33}S-sulfate and ^{3}H-proline, whereas low frequencies have little effect). Mechanical loading within physiological limits is a regulatory stimulus of anti-inflammatory and anabolic responses. Loading suppresses the expression of matrix metallopeptidase as well as prevents the down-regulation of aggrecan in chondrocytes stimulated by IL-1β. Furthermore, cyclic loading could increase cartilage repair. Mechanical loading of high magnitude leads to inflammation and synthesis of mediators of cartilage destruction (IL-1β, TNF-α). These mediators lead to the expression of proinflammatory genes (inos, cox2, MMP_1, MMP_3 . . .).

In summary, mechanical forces appear to be one of the most important parameters of articular, cartilage in inflammation, degeneration, and regeneration [13]. *The chondrocyte perceives the mechanical environment through complex biological interactions with the matrix where the transfer of information involves different signalling pathways. The periodic strain on cartilage generates fluid flows in the tissue which support transport within the tissue, and induces shear stresses on the chondrocytes as well as the appearance of a streaming potential which can modify its capacity of synthesis. A better understanding of the mechanotransduction phenomena could lead to the development of new therapeutic approaches*.

5. Mechanical Forces and Muscle Cells

The different types of muscle cells fulfil many functions within the organism. The most visible example of the impact of mechanical forces is undoubtedly that of the hypertrophy of the skeletal muscles after intense physical training. Regarding cardiac muscle and smooth muscle, quantification is more difficult even though the response to the mechanical stresses is effective.

5.1. Skeletal Muscle

The skeletal muscles are certainly the clearest example of the role of mechanical forces in thr remodelling process [3]. The skeletal muscle responds to mechanical stimulations (exercise) by increasing its mass. Stretching beyond its normal length causes an increase in size by the addition of sarcomeres. Additionally, in response to a mechanical

stress, the muscular cells can modify gene expression in order to adapt their concentrations of contractile proteins (e.g. muscles of marathon runners which increase the concentration of myoglobin and the capillary density). A special property of skeletal muscles is their adaptability (for example optimisation of efficiency by converting their contractile proteins (myosin proteins) from one isoform to another). A loss of more than 50% of muscle mass was observed in a rat kept without exercise for 24 days. This loss can be explained in three phases: reduction of myofibrillar protein synthesis, increase in protein deterioration, deterioration of myofibrillar proteins. In parallel, sporadic training increases protein synthesis and muscular mass. It is well known that men exposed to weightless conditions during space flights are subjected to skeletal muscle atrophy and in-flight exercise is necessary to keep astronauts physically fit.

From a mechanical point of view, the skeletal muscles respond, at first approximation, to a relation proposed by Hill more than 70 years ago. Other more complex approaches have been proposed by introducing viscoelastic elements or by using the concept of fading memory or even on the basis of thermodynamic concepts, but if these theories are interesting, none, to date, has provided a constitutive equation which perfectly represents skeletal muscle.

5.2. Vascular Smooth Muscles

Vascular smooth muscles cells (VSMCs) are the main stromal cells of the vascular wall which assume physiological structural functions (capacity of withstanding the pressure of blood, synthesis of the extracellular matrix,, etc.). In vivo, VSMCs are subjected to one main force (periodic parietal deformation) in relation with blood pressure. The pressure acts perpendicularly to the wall and induces flow across the matrix. This radial flow transports soluble substances from the blood. In other respects, VSMCs are highly sensitive to mechanical stress. Physiologically speaking, VSMCs are the only cell type of the media layer which is an avascular tissue. The myogenic response (arterial contraction in response to an increase in blood pressure) was described a century ago by Bayliss (see [87]), conversely, a reduction in pressure brings about vasodilatation. In both cases, the vascular response occurs in a few seconds. This response is particularly significant in tissues or organs where the speed of perfusion must be as constant as possible (e.g. the brain) [4]. The origin of the myogenic response is related to smooth muscle cells which generate the forces leading to dilation or contraction of the vessels [87]. It has been suggested that the endothelial cells could play the role of "pressure sensor" but this hypothesis has not been confirmed.

Other hypotheses have been proposed:

- Smooth muscle cells are directly sensitive to a change in pressure (stretching of the vessel wall). The activation of certain ion channels by stretching has been demonstrated.
- The tension T on the vascular wall is thought to be proportional to the pressure and the radius of the vessel and inversely proportional to the thickness of the wall.
- The smooth muscle cell is stimulated by flow through the vascular wall linked to changes in intravascular pressure.

In vitro, the impact of the mechanical stresses on smooth muscle cells was and is the subject of many works because these cells are easier to obtain than the cardiac or skeletal muscle cells. Smooth muscle cells subjected to shear stress release mitogenic factors such as PDGF molecules and modulate the production of prostaglandins (PGE2 and PGI2 increase with shear stresses ranging from 0.5 to 2 Pa). It was showed that smooth muscle cells of rabbits subjected to 10% stretching at a frequency of 1 Hz synthesised three times more type I and III collagens and hyaluronate. Studies on cyclically stretched parts of pig aortas also showed increased synthesis of desmin and vimentin, two components of the cytoskeleton. This work is generally carried out over periods of several days. However, some studies were carried out by fluorimetry on the intracellular calcium variations in the seconds that follow abrupt stretching showed an increase of these for several minutes. This effect is increased by the addition of extracellular calcium and inhibited by EGTA. In other respect chronic changes (i.e. hypertension) in local mechanical stresses cause vascular remodelling and modification of the mechanical properties of the artery.

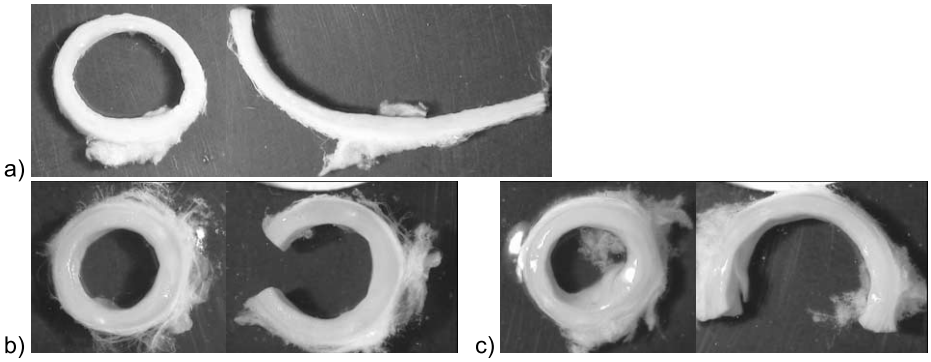

Figure 1. Images of rings of tested human segments of arteries before (unloaded) and after (zero-stress) radial cut. **a)** Healthy specimen with $h_u = 1.52$ mm, $D_u = 10.55$ mm, opening angle $\alpha = 132°$. **b)** and **c)** Atherosclerotic specimen with $h_u = 1.65$ mm, $D_u = 8.3$ mm, opening angle $\alpha = 23°$ (radial cut opposite to atherosclerotic plaque) and $\alpha = 64°$ (radial cut inside plaque). [Personal results – Blondel et coll. IEEE Transaction on Biomedical engineering – 2001; 48:442–451].

Another important parameter for the analysis of stress – strain in vascular remodelling, is the zero stress state (Fung, [33, 34]). For a vascular blood vessel, this state can be experimentally shown by cutting a slice of vessel perpendicular to the axis (Fig. 1). The section will open up on its own because of the release of residual stresses which can be characterized by the opening angle. The opening angle varies with the location in the vascular tree. If a body is in a zero-stress state, the cut will not cause any opening. The zero stress state varies with the location in the vascular bed and is modified in different pathologies (diabetes, hypertension, atherosclerosis, etc.).

5.3. Cardiac Muscle

Mechanically, the difference between cardiac and skeletal muscles lies in the importance of resting tension in the normal function of the heart which determines the end diastolic volume. It is well known that hypertension increases the cardiac volume and also causes morphological changes in cardiomyocytes. However, the stress-strain relationship of a hypertrophic myocardium is no different from that of normal tissue. In addition to increased cell volume, blood pressure overload results in stimulation of the second messenger pathway and the expression of specific genes by myocytes (c-fos, c-jun, c-myc) as well as shock protein 70 and changes in the β myosin heavy chain.

The term myocardial remodelling concerns all the states in which the structure of the heart is modified (hypertension, infarction, etc.). The observed adaptation remains an incomplete process of regeneration. Remodelling linked to adaptive changes in the expression of genes, can be of mechanical origin. Thus, the stretching of the myocyte is a parameter of activation of transcription as well as of protein synthesis. However, many complex channels lead to permanent modifications in which intracellular calcium doubtless plays a determining role. At the same time, a rearrangement of the microtubular network has also been observed. Since there are a numerous genes that can be involved in remodelling, it is still difficult to identify the ones of interest. However, these studies highlighted a significant modification in the genome during the remodelling phenomena.

6. Mechanical Forces and Endothelial Cells

In vivo, the vascular endothelial cell (EC) is subjected to three types of mechanical forces whose intensity vary according to the vascular bed: shear stress (τ, some mPa), hydrostatic pressure (p, some Pa) and periodic parietal forces leading to deformation under cyclic load between 0 to 3 Hz. The shear stress (τ) induced by the blood flow acts tangentially on the EC. It is generally described by the relation of Poiseuille. The hydrostatic pressure acts perpendicular to the surface of the endothelium. It affects the extracellular matrix as well as the endothelium.

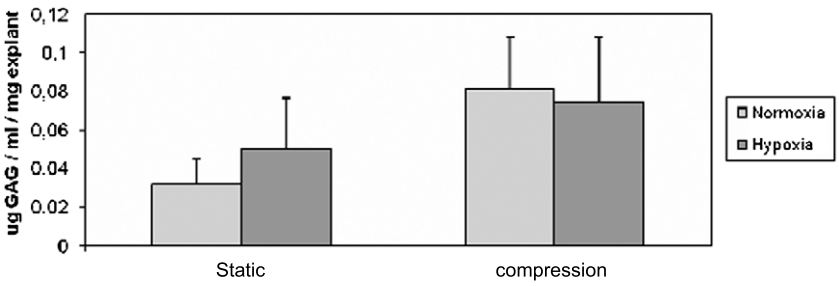

Figure 2. Influence of compression on GAG synthesis by cartilage in Hypoxia end Normoxia (Personnel results).

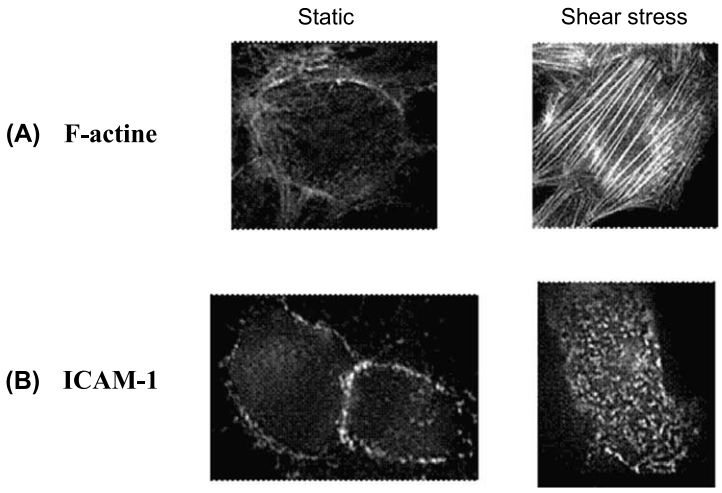

Figure 3. Influence on HUVEC of a shear stress (0,2 Pa/4 hours) on F-actine (A) and ICAM-1 (B) (Personal results).

The response of the EC to mechanical forces has been widely studied and variations in a large number of cell functions have been reported (electrophysiology, biochemistry, receptors, regulation of gene, etc.) [27, 35, 68, 72] The various responses of EC to mechanical forces can be classified according to reaction time, although they are often simultaneous. Different authors described modifications of the vascular endothelium in relation to the wall shear stress. In 1981, Dewey and al. [25, 28] showed the dynamic response of EC in relation to shear stress, and Nieponice and al. [63] suggested that the morphology of the EC could be an indicator of local hemodynamic conditions. The morphological changes corresponding to the lengthening and the orientation of EC parallel to the direction of the flow and the reorganization of the cytoskeleton are responsible for motility and cell adhesion. With low shear stresses, in vivo and in vitro, ECs are polygonal and are lengthened more than ECs subjected to a high shear stress. In addition, the morphological changes in ECs vary according to their localization in the vascular bed and flow conditions (laminar periodic, disturbed flow, etc.). In the last 20 years, the influence of local hemodynamic conditions on the EC has been the subject of increasing interest. In other respects, the pulsatile nature of blood flow causes cyclic stretching of EC. Stretch deformation has been observed in deformable substrates with cyclic uniaxial and biaxial stretch.

All the elements of the cytoskeleton (actin filaments, microtubules, intermediate filaments, etc.) undergo reorganization during the application of shear stress, influenced by both the amplitude and the period. Proteins related to the cytoskeleton, such as the vinculin, Zyxin, VASP (vasodilatator stimulated phosphoprotein) are associated at the ends of actin filaments to the periphery [35, 72]. Following prolonged shearing, ECs are oriented in the direction of the fluid flow. In cells, the stress fibres and microtubules, as well as the intermediate beam alignment of the filaments are aligned according to the direction of the fluid flow (Figs 2 and 3). This reorganization of the

cytoskeleton has a direct effect on cell signalling, in particular on protein phosphorylation (such as the VASP), or enzymes involved in mechanotransduction [20]. It also influences the properties of the endothelial cells (EC), such as adhesion, release of the Von Willebrand factor,... It was also observed that EC activation involves increased expression of adhesion molecules such as ICAM-1, with firm adhesion. In addition, during the application of a shear stress, ICAM-1 migrated towards the apical pole of the EC via the cytoskeleton and was accessible to circulating adhesive cells (e.g. leucocytes). Using fluorescence recovery after photobleaching (FRAP) Chien et al. [22] observed *in vitro* modification of membrane lipid fluidity which can be a factor causing a modulation of the conformation or interaction of membrane proteins. In other respects endothelial monolayer of HUVECs exposed to non-uniform shear stress (like in a vascular bifurcation) produce change in cytokine and chemokine profile (↗ IL-8 and MCP-1 release) [23]. These observations underline that non uniform shear stress induces secretion of chemoattractant molecules that can promote the recruitment and migration of leukocytes across the atherogenic regions of the vessel wall.

Cyclic uniaxial stretching induces orientation of cells and stress fibres in a direction perpendicular to the stretch direction. With biaxial stretch, no specific orientation of cells or of stress fibres was observed. It was also observed that uniaxial cyclic stretch results in a transient activation of JNK with returns to control level after 6 hours and that a biaxial stretch induces an activation for 12 hours or more (Chien [23]).

Regulation of gene expression of molecules synthesized by the EC, like ET-1, PAF, PDGF A and B, MCP-1, adhesion molecules (ICAM-1, VCAM-1), is influenced by the fluid flow [11,22,23,28,46,65,85,86]. Thus, expression of PDGF-B and FGF is increased in the EC and in the vascular smooth muscle cells when these are subjected to shearing forces. A gene coding for ICAM-1, which is involved in inflammation, increased when EC were subjected to shearing forces. Expression of VCAM-1 and ELAM-1 was not affected under the same conditions. The other transcription factors responsible for the activation of promoters by shear stress are the nuclear factor kappa B, the activating protein-1 (AP-1), the early-1 growth promoter (Egr-1) c-fos, c-jun, c-myc and stable (Sp-1). The variations observed in gene regulation by microarray analysis suggest that two types of gene elements that are sensitive to shear stresses (positive and negative) may exist. For instance, constant shear stress regulates atherogenic genes (egMCP1) but non-constant shear stress causes sustained activation of MCP1. This result provides an explanation for the observed localization of atherosclerosis lesions.

In summary, the mechano-sensing of EC is multifactorial, and the application of shear stress can lead to the activation of various signalling pathways. It was demonstrated that integrins can sense shear stress, but other membrane components involved in the sensing of mechanical forces include G protein receptors, ion channels, membrane glycocalyx and lipids. Mechanotransduction phenomena involve numerous sensors and genes. In another respect, if endothelial cells react to various mechanical forces, it should be recalled that the response times vary from a few seconds to 24 hours, depending on the parameters concerned. Thus NO synthesis takes place in seconds, that of PG in minutes whereas it takes several hours to observe the rearrangement of the cytoskeleton and an alignment of the cells in the fluid flow suggesting that multiple complex mechanisms are involved (Fig. 4). Many of these changes appear to involve gene regulation at the transcriptional level and are analogous to EC activation by cytokines and the endothelial lining of the vascular system is a dynamic interface which responds to humoral mechanical factors.

7. Conclusions

The impact of mechanical forces (on cells mechanobiology) is now acknowledged to better understand the synthesis of the extracellular matrix, the secretion of specific molecules or the induction of specific functions by intercellular communication. However, many mechanisms still need clarifying and many unknowns remain. Understanding the phenomena of transduction of mechanical signals should enable a better approach to physiopathological phenomena (osteoarthritis, atherosclerosis, etc.) as well as the development of regenerative therapies via cell therapy or in vitro preparation of biotissues with the help of modified or unmodified stem cells including myocardial grafts, preparations of cartilage, bone or small diameter arteries.

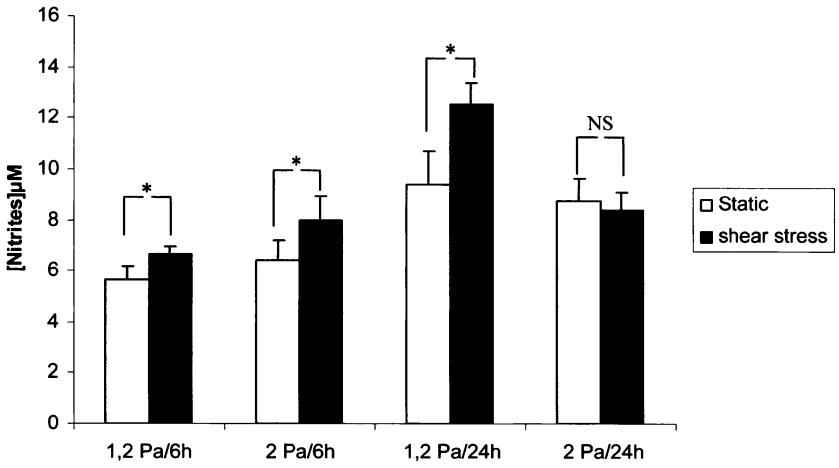

Figure 4. Influence of laminar shear stresses on NO synthesis by HUVECs Supernatant was collected and nitrite level in the medium was measured using the Griess reagent kit. (Personal results A. Kadi et al., Clinical Hemorheology and Microcirculation 2007; 37, p136).

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