MECHANOBIOLOGY AND CARTILAGE TISSUE ENGINEERING*

Céline Huselstein[1], Natalia de Isla[1], Jing Wei Yang[1], Yun Wang[1], Jean-François Stoltz[1, 2], Sylvaine Muller[1]

1 (Mécanique et Ingénierie Cellulaire et Tissulaire, LEMTA UMR INPL CNRS 7563 et IFR 111 Bioingénierie, Faculté de Médecine, 54505 Vandoeuvre-lès-Nancy Cedex, France)
2 (Unité de Thérapie Cellulaire et Tissulaire (UTCT) – CHRU – 54500 Vandoeuvre-lès-Nancy Cedex, France)

ABSTRACT

The cartilage is a hydrated connective tissue in joints that withstands and distributes mechanical forces. Chondrocytes utilize mechanical signals to maintain tissue homeostasis. They regulate their metabolic activity through complex biological and biophysical interactions with the extracellular matrix (ECM). Although some of the mechanisms of mechanotransduction are known today, there are certainly many others left unrevealed. Different topics of chondrocytes mechanobiology have led to the development of tissue engineering. It is the concept of substitute tissue developed *in vitro*, from bioresorbable or non-bioresorbable scaffolds and from cells harvested in a physiologic mechanical environment.

This study constitutes an overview of both chondrocytes mechanobiology and cartilage tissue engineering.

1. INTRODUCTION

It is now accepted that the organization of cartilage, bone, blood vessels, heart, muscles... is under the control of regulatory genes which mediate the expression of downstream genes and that the mechanical forces on fetal tissues are fundamental on musculoskeletal system growth and differentiation[7, 11]. While the biological effects of mechanical forces on various cell types (en-

dothelial cells, chondrocytes…) are now well reported, the molecular mechanisms being able to explain these phenomena are still poorly understood and it is often difficult to explain the transition between a mechanical stimulus and its physiological response. Concerning specifically the cartilage, we know now that the ratio proteoglycans/collagen is essential to understand its particular mechanical properties which enable the cartilage to resist to the great variations of compression forces[3]. In addition, the presence of proteoglycans-associated negative charges results in elevated concentrations in mobile cations and in an acid hyperosmolar environment for the chondrocyte. More, the biological effects are dependent on the type of compression. Thus, a static compression can reduce GAG and collagen production while a cyclic compression can induce a reverse effect.

As a matter of fact, mechanical forces exerted in vivo onto the joints during these movements (0 to 20 MPa at the hip site) are the result of a complex combination between tension mechanisms, stress and compression forces, this last phenomenon being the most important within the cartilage[1]. The chondrocyte is particularly reactive to these forces which could ultimately alter its metabolism and therefore the mechanical properties of the extracellular matrix and of the cell-extracellular matrix interactions (focal adhesion mechanisms). Thus, a motionless cartilage can lose its mechanical resistance properties and an excessive strain on cartilage, without any resting phase, could accelerate degeneration. Fundamentally, it is today well accepted that the chondrocytes of the articular cartilage mediate their metabolic activities through mechanical signals. The application of a mechanical force, such as pressure, will specifically result in chondrocyte deformation and in stimulating cellular and sub-cellular events, thus interfering with the cell physiology. The magnitude of the characteristics (rate, time, and intensity) of this mechanical force on chondrocyte properties such as its differentiation, its ability to synthesize the extracellular matrix or such as the cell stimulation is now demonstrated.

Articular cartilage restoration is problematic due to its poor capacity for repair once damaged or diseased. Tissue engineering of articular cartilage is a promising alternative for cartilage repair. Cell, biomaterial scaffolds, biochemical and physical regulatory signals can be utilized to engineer cartilage *in vitro* and *in vivo* (Figure 2). During the last years, investments have increased remarkably.

2. CHONDROCYTES MECHANOBIOLOGY

The perception of mechanical signals and biological responses to these stimuli are fundamental properties of load bearing articular cartilage in diarthrodial joints[6].

Fluid expression alters the ionic environment by concentrating extracellular cations (Ca^{2+}, H^{+}, Na^{+}) and hence alters the electrochemical gradient across the cell membrane. Stretch and flow-induced dynamic compression can open channels allowing ingress of Ca^{2+} and other ions. Such changes in the intracellular concentrations of signaling

molecules could have a rapid influence on cellular metabolism (Figure 1)[14, 17].

At the cell surface, matrix receptors link the ECM to the cell interior through elements of the cytoskeleton and other component proteins of signal transduction pathways. Among these receptors, articular chondrocytes have been shown to express members of the integrin family. Under mechanical stimulus, integrin mediated adhesion to ECM protein can activate several cytoskeleton associated protein, such as paxillin, tensin and intracellular signaling protein like FAK (focal adhesion kinase). FAK has emerged as a key signaling component at focal adhesions. It is activated *via* autophosphorylation at tyrosine 397 that is initiated by integrin engagement with its ligand. Integrin signaling is coordinated with both the organization of cytoskeleton and with signaling by growth factor and cytokine receptor (Figure 1)[9,16].

3. CARTILAGE TISSUE ENGINEERING

As far as cartilage is concerned, developing biocartilages through engineering is a full growing field. Various approaches have been proposed according to the type of cells (chondrocytes or mesenchymal stem cells) and to the matrix support.A good knowledge of cells-matrix interactions according to the applied mechanical forces will be required.

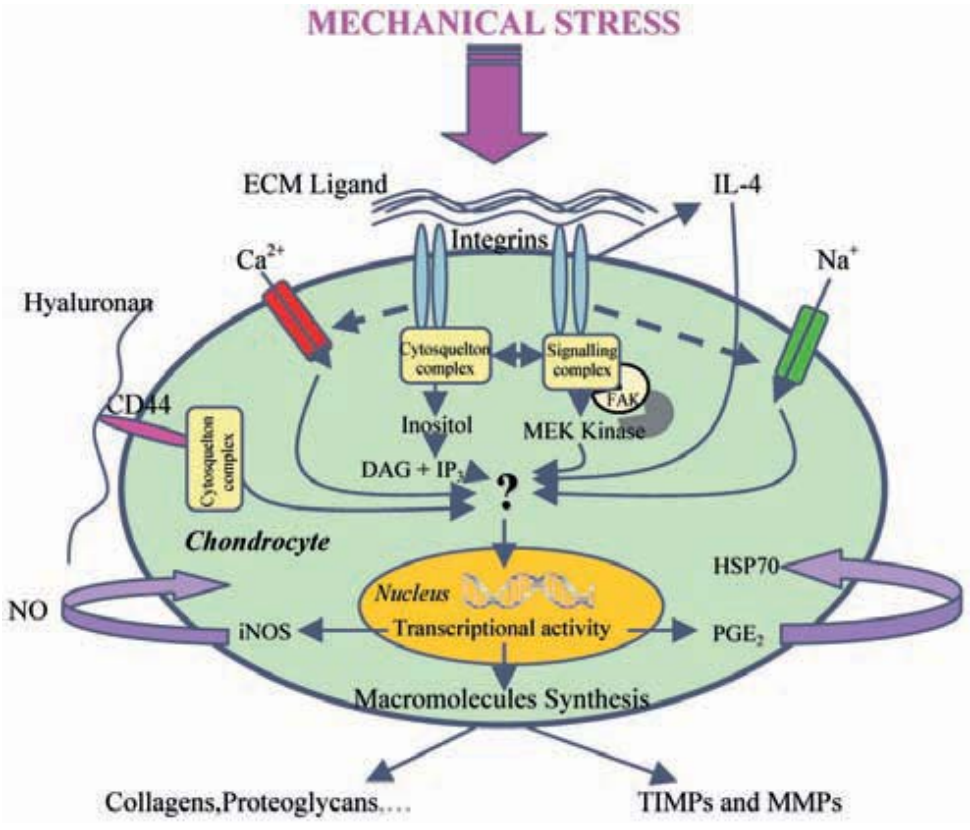

Fig.1 – Schematic representation of chondrocyte mechanobiology[9]

3.1. Cell, biomaterial scaffolds and biochemical conditions

Thus, it has been shown that implanting chondrocytes-including biomaterial improves cartilage injury in animals. It has been proposed in man as soon as 1994 to use adult chondrocytes obtained via culture from cartilage tissue[4,5]. However, on the one hand using mature adult cells from a single layer culture exhibits a moderate potential of proliferation able to induce a dedifferentiation of the chondrocyte into a fibroblast and on the other hand requires collecting healthy cartilage in biopsy samples. Besides, the presence of fibrocartilage seems to be predominant. Recently, various reports have shown that mesenchymal stem cells collected from bone marrow could differentiate in vitro in adding TGF β, d'IGF, BMP2 or FGF into chondroblasts, able to synthesize type II collagen[15]. Thus, given their capabilities of differentiation, mesenchymal stem cells seem to be the most interesting way to collect cartilaginous cells.

Other components seem also to play a role in the differentiation of these cells into chondrocytes. Indeed, cartilage is a non vascular connective tissue, able to react to the mechanical requirements of the locomotive apparatus, the restoration capacity of which is however poor. The permanent pressure forces impacting on this system are an essential regulatory factor of its functionality[8]. Chondrocytes synthesize an extracellular matrix containing various types of collagen, initially a type II collagen then later on a type X collagen, proteoglycans (more specifically aggrecan and hyaluronates [HA]). Structural and functional integrity of cartilage is kept stable through the quantitative and qualitative maintaining of these components. In addition, of note, the fact that this extracellular matrix is in contact with the synovial fluid, the redox state of which, as well as the content in ionic charges and proteins, could influence chondrogenesis. Thus, osteoarthritis or any other inflammatory condition of the synovial fluid could result in pro-inflammatory cytokines release such as Il-1 the effect of which is negative on cartilaginous synthesis[10,13]. Inversely, hypoxia seems to be beneficial.

Recently some authors have shown that mesenchyumal stem cells differentiation into chondroblasts was even more efficient when mechanical forces were applied onto the cells[11,12]. To do so, TGFβ-cultivated mesenchymal stem cells were laid down on 3D containing-hyaluronate gel matrix and submitted or not to a daily compression for several weeks. After 3 weeks, results showed that the applied mechanical force could accelerate the differentiation of mesenchymal stem cells into chondroblasts validated by an increased synthesis in aggrecan and type II collagen.

Various synthetic, organic supports or hybrid biomaterials have been proposed in cartilage engineering. Among synthetic or biological components are polylactic acid and polyglycolic acid polymers, collagen-based material, fibrin or polysaccharide polymers such as hydrogels[12]. Hydrogels are composed of reticulated polymers able to absorb a great amount of water. Mechanistically, hydrogels have the advantage to use water like cartilage. Under compression, water is released from hydrogel, allowing hydrogel to absorb

a strain ; once this strain over, water can come back to its initial place within the material and this latter returns to its initial volume. Biologically, hydrogels have a sufficient 3D porous environment to allow cellular proliferation as well as nutriments transportation. Among hydrogels, sodium alginate hydrogels are a standard in cellular morphology trials, in proteoglycans and collagen synthesis trials, as well as a standard as a natural component of extracellular matrix. It has a similar structure to cartilaginous glycosaminoglycans and has been proposed as an interesting material to sustain the chondrocyte phenotype[9]. Recently, a MIT research group has developed an injectable polysaccharide-based gel integrating a photosensitive molecule able after injection to be photopolymerized by UV radiation.

In summary, as far as cartilage engineering is concerned, one of the major issues will be to have at our disposal a biomaterial with optimal mechanical characteristics and able to integrate the targeted cells (Figure 2). Many research works must be developed in order to characterize the optimal biocartilage.

3.2. Physical regulatory signals

Lastly, mechanical loading applied on tissue constructs may play an important role to manufacture constructs that mimic native extracellular matrix. Numerous physical factors have been utilized to modulate tissue engineered cartilage, including fluid flow, dynamic fluctuations in hydrodynamic shear and pressure,

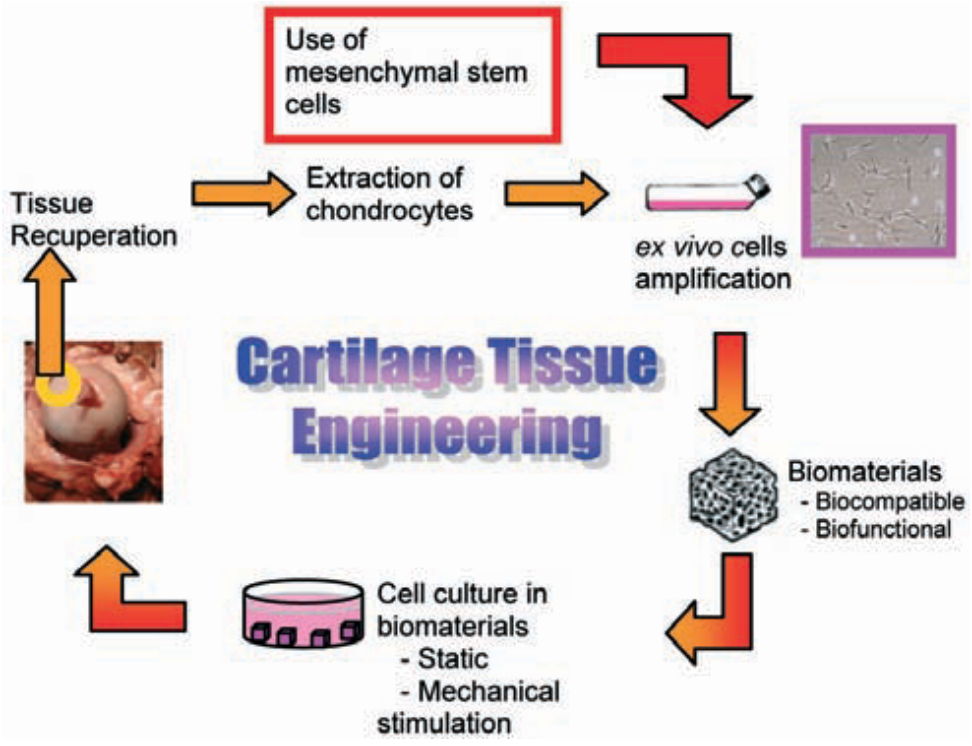

Fig. 2 – Basic principles of cartilage tissue engineering

cyclic hydrostatic pressure, cyclic mechanical compression and cyclic stretch. Although the effects of loading on extracellular matrix synthesis in cartilage tissue have been investigated a lot, there has been a lack of systematic effort to find the optimal ratio between loading and rest for the most efficient matrix synthesis.

CONCLUSIONS

Different possibilities seem now to be available to manufacture a biocartilage. Most of the above concepts are not really new, but the recent advances in cell biology, polymers, genomics, synthesis and surface processing are encouraging. In addition, the characterization of biocartilage must go along with a better knowledge of the impact of mechanical forces upon the various pathways contributing to the cartilage synthesis and directly involved in the inflammation process and its inhibition.

The main requirements to fulfil cartilage manufacturing are the following: a 3D scaffold allowing consistent cell attachment, maintenance of cell phenotype and in vitro optimisation of cell culture in a biochemical environment. All our studies must be aimed at improving cartilage morphogenesis and in vitro tissue cultures in view to develop clinical and pharmacological applications.

* This work was partly supported by region de Lorraine (CPER) and French embassy in Beijing.

** Corresponding author:
Celine.Huselstein@medecine.uhp-nancy.fr

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