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Review

Cartilage biomechanics: A key factor for osteoarthritis regenerative medicine

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ABSTRACT

Osteoarthritis (OA) is a joint disorder that is highly extended in the global population. Several researches and therapeutic strategies have been probed on OA but without satisfactory long-term results in joint replacement. Recent evidences show how the cartilage biomechanics plays a crucial role in tissue development. This review describes how physics alters cartilage and its extracellular matrix (ECM); and its role in OA development. The ECM of the articular cartilage (AC) is widely involved in cartilage turnover processes being crucial in regeneration and joint diseases. We also review the importance of physicochemical pathways following the external forces in AC. Moreover, new techniques probed in cartilage tissue engineering for biomechanical stimulation are reviewed. The final objective of these novel approaches is to create a cellular implant that maintains all the biochemical and biomechanical properties of the original tissue for long-term replacements in patients with OA.

1. Introduction

One of the main objectives in regenerative medicine is to generate a native-similar tissue replacement for joint diseases. Since articular cartilage (AC) has a significantly poor self-renewal ability, is avascular, aneural and has a complex associated surgery, it constitutes a sought-after target for researchers and clinicians. Currently, there are many different treatments: from nonsteroidal anti-inflammatory drugs (NSAIDs) to invasive interventions including implants, cellular treatments or osteochondral rupture among others. Some of them have achieved meaningful results for patients, though without achieving the levels of quality of life prior to the injury. In addition, the biomechanics of the joint is always deteriorated after the disease, independently of the treatment applied. Because of this, many scientists tried to discover the physical principles that govern AC and how they change in different joint disorders. Until now, this has not been an easy task and there is no consensus about which biomechanical principles are governing the regeneration of cartilage tissue. This review compiles the principles behind the biomechanics of the cartilage (Fig. 1), describing the characteristics of healthy AC and how it changes in osteoarthritis (OA), the most common cartilage disease. In addition, we describe previous studies about the application of biomechanics fundamentals into regenerative therapies of AC. Biomechanics is a ground part of cell biology and requires interaction with biochemical pathways and cell metabolism. Consequently, optimizing biomechanics and the biochemical niche as an interconnected system can potentially contribute to the consecution of a real long-term viable replacement for can overcoming OA.

2. Cartilage

Cartilage is a flexible, avascular, aneural and alymphatic connective tissue. Its constituent cells, called chondrocytes, receive nutrients through diffusion from the synovial fluid, which is rich in proteins derived from the blood plasma, and from the joint tissues (hyaluronic acid, PRG4) [1].

Articular cartilage (AC) is a specialized form of hyaline cartilage with a thickness of 2–4 mm. The ECM of AC has the capacity to retain

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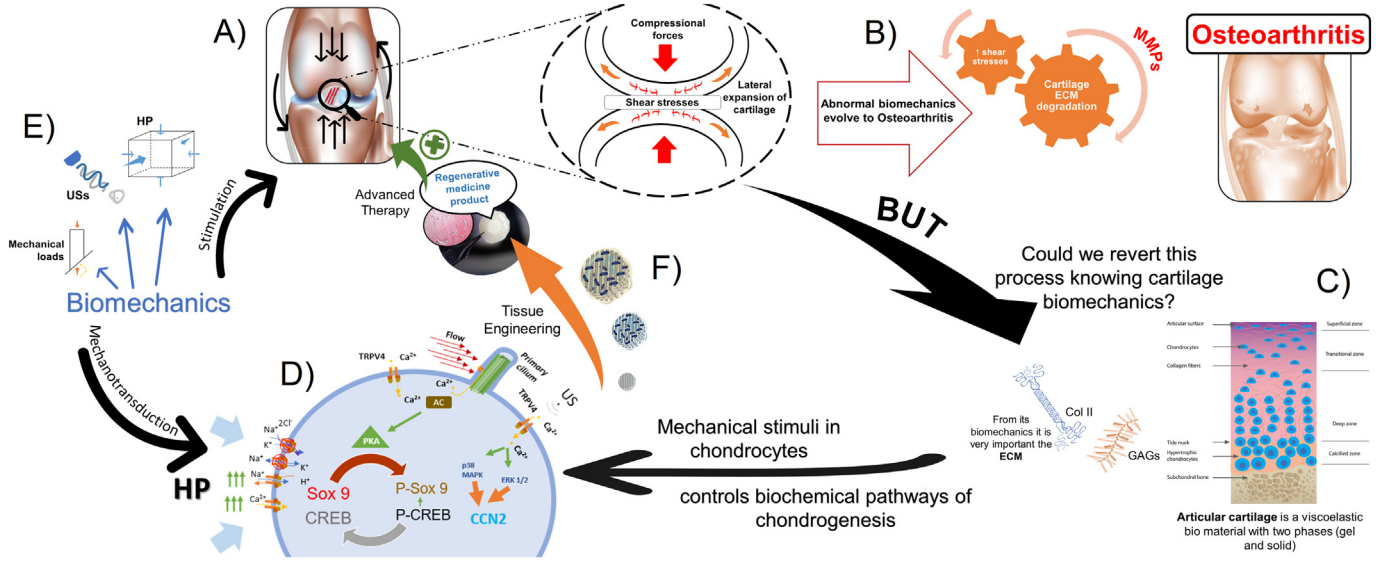

Fig. 1. Schematic representation of the key role of biomechanics in development of OA and the therapeutic strategies including these physical aspects useful on cartilage regenerative medicine. A) Any variation in normal joint behavior induces changes in physical factors such as compressional factor and shear stresses between adjacent bones induces that produce abnormal biomechanics that is involved in OA development. B) An increase in shear stresses will induce an increase on friction and strain which is translated in a degradation of the ECM of the AC in early stages of OA. C) The ECM (mainly composed by collagen type II and GAG) is essential to maintain the viscoelastic characteristics and the well-function of the AC, which at the end is in charged to reduce joint friction and to cushion our steps. D) This ECM is tailored by chondrocytes that are sensitive to mechanical stimuli, *i.e.* they have lots of mechano-sensors which can detect small variations of surrounding physical cues and change their metabolism as function of them. With this in mind, thanks to a precise knowledge about how controlling biomechanics pathways to induces chondrogenesis, OA could be reverted. E) The comprehension of how external physical stimuli (biomechanics) such as mechanical loads, ultrasounds, hydrostatic pressure can interact both in molecular pathways and in damage regions stimulating the tissue regeneration. F) To promote and to enhance these biomechanical reparative processes, tissue engineering applied to OA can generate new regenerative medicine products useful in advanced therapies that fulfilled all the biomechanical and biochemical properties of healthy AC.

high quantities of water due to its abundance of sulfated glycosaminoglycans, that possess strong hydrophilicity and negative charges [2]. This property is intrinsically connected with the main function that AC has, namely allowing movement without friction and counteracting the impact of compression forces applied onto the joint [1]. Given that AC is a viscoelastic "composite" dominated by two phases (gel and solid), it can respond to mechanical stimuli in two different ways: i) by deforming the porous matrix, which implies an increase in the number of contact points and a decrease of contact stresses; ii) by releasing interstitial fluid through the porous matrix consequently raising the lubrication of AC [3].

Concerning biomechanics, a deep comprehension of the ECM components of AC is required beforehand. Type II collagen in the form of cross-linked microfibrils has been shown to form over the ECM. These fibrils make connections with other tissue-specific collagens of the cartilage, such as types IX and XI collagen among others (type VI, X, XII and XIV). Although the latter collagen types are almost insignificant components of the ECM in proportion, it seems they have a crucial role in the biomechanical behavior of the whole structure [4].

Other important molecules involved in the composition of the ECM are the proteoglycans, mainly aggrecan and, in lesser amounts, biglycan, decorin and others (*e.g.* fibromodulin, lumican) [5]. These uncommon proteoglycans are involved in the arrangement of the AC natural structure thanks to several interactions with collagen II alongside with Transforming Growth Factor (TGFβ) and keeping the fixed charge density constant which regulates water concentration [6].

Aggrecan is involved in the two principal functions of the cartilage from a biomechanical point of view: i) Together with other molecules (*i.e.* chondroitin sulfate), it modulates the fluid pressurization of the tissue thus the structure can be maintained and the articular surface resists deformations allowing a major lubrication; ii) the concentration of aggrecan increases through the superficial zone and this gradient is correlated with the amount of extracellular water retention that inhibits external compressions [7].

3. Osteoarthritis: a biomechanical disease

OA (Fig. 1A) is by far, the most representative degenerative disease related with the joints. It has been estimated that 250 million people worldwide suffer from of knee OA (2012), being a major cause of pain and disability in adults [8]. The Global Burden of Disease (GBD) estimated that OA approximates 0.6% of all disability-adjusted life-years (DALYs) and 10% in musculoskeletal conditions [9]. The pathological pathway leading to OA consists on a chronic low-grade degradation of AC (Fig. 2), which is the major driver of ongoing joint degeneration [10]. In such a way, OA should not be considered as a disease but as a common end of multiple secondary pathways related with aging, possible traumas, obesity and their correspondence altered biomechanics of the joint [11]. More and more researchers have supported this idea, which in principle could seem ambitious, during the last years. Ganz et al. in 2008 first introduced the suggestion that the early steps of OA process are related to biomechanical aspects of the cartilage tissue (Fig. 1B) [12], and recently, other authors have experimentally confirmed this statement [13].

Inside the biomedical research community, it is globally accepted that biomechanical properties of the tissue behave as function of the ultrastructural organization which depends on the biochemistry and cell-cell and ECM-cell interactions [14] to such an extent that any small alteration in these properties will drastically alter tissue biomechanics [15]. The main axis of the development of OA is a precedent of mechanical derangement that produces a low-grade damage in the AC [16]. Thus, from the biomechanical point of view, three different stages can be established in OA development: i) the proteolytic breakdown of the ECM, ii) the fibrillation and erosion of the cartilage surface and, iii) the beginning of synovial inflammation (Fig. 2) [17].

Together with biomechanical stresses, biochemical and genetic factors participate in the progression and development of AC in OA. They contribute to decomposing chondrocyte-ECM interactions, in turn modifying cell metabolism [18]. Matrix gene expression of

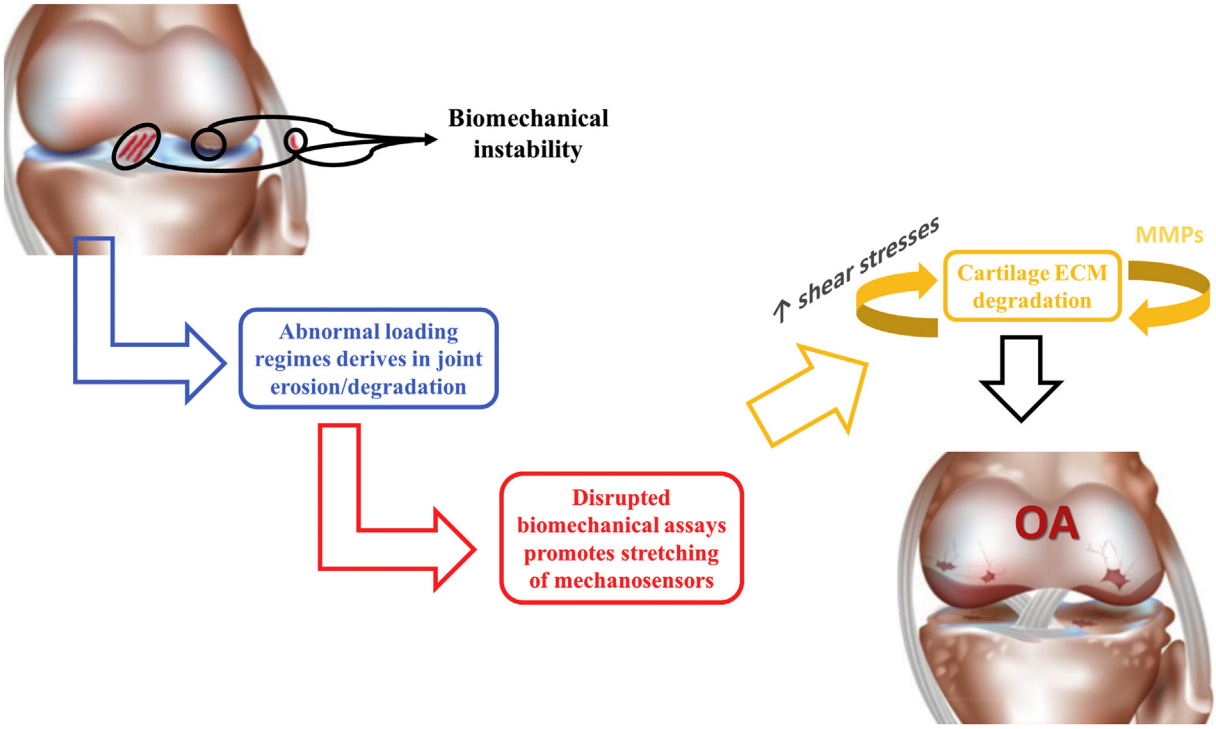

Fig. 2. Cascade of biomechanical events in OA. The original biomechanical instability (*i.e.* a small scar or just an unalignment of the joint) promotes the proteolytic breakdown of the ECM. The degradation of this ECM induces the stretching of the mechanosensors involved in normal AC homeostasis. An abnormal homeostasis of AC will degrade more the ECM and develop the synovial inflammation (which induces MMPs routes).

chondrocytes is altered in OA as it presents collagen molecules (type X, III, VI) that are normally missed in adult normal AC [19].

In the initial stages of OA, chondrocytes tend to repair the matrix loss with no positive result since the synthesis of catabolic cytokines and matrix degrading enzymes increases [20]. Unfortunately, it induces the leakage of proteoglycans and the breakdown of type II collagen, which start at the cartilage surface. Consequently, the water concentration increases implying a critical reduction of the tensile strength of the ECM [18]. Other important enzymes involved in the degradation of articular cartilage in OA are the matrix metalloproteinases (MMPs). Previous studies have shown that tissue specific MMPs 1,8 and 13 (collagenases I, II and III, respectively) are involved in OA. The posterior step is aggrecan degradation produced by aggrecanases 1 and 2, which are family of the ADAMs (a desintegrin and metalloproteinases) attached to type 1 thrombospondin (TS1) [21].The cascade of initial steps results in a partial or total degradation of the AC (Fig. 2), that is in charge of reducing articular erosion. Therefore, shear stresses produced by friction between the adjacent bones of the articulation increases [22]. Growth of bone spurs thereby progresses during disease development, which will lead to articular inflammation and joint pain [23]. In this state, the presence of interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α) [24] induces the synthesis of other inflammatory factors like cyclooxygenases (COX-1, COX-2) [25], the phosphorylation of mitogen-activated protein kinase (MAPK) [26], as well as the degrades I-kBs activating Nuclear Factor-Kappa B (NF-kB) [27].

4. Key mechanical factors in cartilage tissue engineering of OA

In the last decades, several tissue engineering (TE) cartilage products like the matrix-associated autologous chondrocyte implantation (MACI), Hyalograft® C, NeoCart®, NOVOCART® 3D, Cartipatch®, *etc.* have tried to mimic articular cartilage [28]. But, current bioengineered neocartilage is far from being optimal in comparison with its mature counterpart (Fig. 1C). In part, this is because it is a challenge to create a construct that collects the anisotropy and homogeneity in its structure giving rise to the characteristic mechanical properties of the AC [29]. Thus, the main challenge for taking the tissue engineered cartilage (Fig. 1F) to the clinic is to design biomechanical properties of the final implant which are close to native tissue. In addition, autologous chondrocytes are not the best cellular source for making this autologous explant, since the percentage of these cells inside the articular cartilage is < 5% among other drawbacks. In addition, during the time of *in vitro* expansion, monolayer cell cultures present an overexpression of type I collagen and versican in lieu of type II collagen and aggrecan production [30]. This process by itself, results in a reorganization of the microfilament structure of the three-dimensional (3D) ECM, implying that biomechanical stresses of the microstructure can change, which are crucial for the correct tissue performance [31].

On the other hand, mesenchymal stem cells (MSCs) have demonstrated a real potential in differentiating healthy chondrocytes [32]. In addition, MSCs promote the resilience of chondrocytes when they are co-cultured *in vitro* [33]. But not everything in the field of MSCs is an advantage. Nowadays, there is no stablished cell therapy approach approved for therapeutic interventions [34], although, the use of MSCs derived from the umbilical cord for AC treatment has been approved within the last year. Even more, the differentiation potential of MSCs is age-dependent, being a limitational factor for autologous implants [35].

Among all possible mechanical stimuli applied to cells (Fig. 1E), the most reliable ones are hydrostatic pressure, direct compression and application of fluid shear stresses. In the case of cartilage TE, the application of physical patterns can stimulate the synthesis of type II Collagen and glycosaminoglycans (GAGs), and promote chondrogenesis [33].

4.1. Mechanotransduction

Currently, it is a well-known fact that mechanotransduction provides many of the biochemical and biomechanical cues influencing cell migration, phenotypic modulation and cell survival. Even more, cell-cell interactions also contribute with long-term cooperative mechanism

of orientation in similar biomechanical environments (*i.e.* small variations in Young's modulus) [36].

The influence of biomechanical stimuli in chondrocyte cell fate is a complex process that even today is yet to be fully understood (Fig. 1D). The area that studies this aspect is mechanobiology, and scientists call these physical interactions as mechanotransduction. Mechanotransduction can be considered as any pathway in which a transduction signal from a mechanical stimulus into electrochemical activity is involved [37]. Mechano-sensitivity is a general property of cells, as well as voltage sensitivity and thermal sensitivity among others; consequently, physical interactions are common events in the cell cycle.

Mechano-sensors (proteins) present in the cell membrane, and they are mostly involved in all mechanotransduction phenomena. This is the case of talin, a critical protein in the correct functioning of focal adhesions (FA). Talin undergoes an extensive conformational change triggered by the generated force of the actin myosin complex, which in turn generates a cascade of events where the vinculin, the α-catenin, *etc.* take part [38]. Other well-known mechano-sensors are integrins: transmembrane proteins that are strongly involved in the transmission of mechanical stresses along cell plasma [39].

4.2. Hydrostatic pressure

The fact that AC is a highly hydrated tissue (70–80% of water content) means that on the application of an external compression, the entrapped water will “resist” the deformation. Translated to the cells, this results in an over-exerted force that will be homogeneous along the whole cell surface producing isotropic deformation, that is hydrostatic pressure (HP) [40]. Regarding the idea of how HP and cartilage interact, diverse studies (Supplementary Table 1) have suggested a direct correlation between the HP stimulation on the cells and the behavior of cell membrane channels [41]. Despite the fact that HP does not imply a measurable deformation in the cartilage tissue, it interacts with the transporter proteins compressing the void spaces created by the folding orientations of these complexes [42].

It has been found that the Na/K pump is dramatically constrained under a static HP load (2.5–5 MPa), or even completely suppressed under 50 MPa [41]. It also inhibits Na/K/2Cl transport activity (Fig. 3). On the other hand, Browning et al. showed that Na/H pump activity was increased, and they also found that HP modulates the phosphorylation of the pump [43]. Mizuno discovered that exercising an HP in the middle zone of the cartilage results in an expansion of the intracellular calcium concentration (Ca^{2+}) due to stretching of activated calcium channels [44].

The usual range of stresses affecting any given joint lie between 3 and 10 MPa, but can reach up to 18 MPa which is the maximum measured stress at the hip joint [45]. In addition, the frequency of these stresses when walking is in the magnitude of 1 Hz in humans [46]. Some authors have studied the relevance of these parameters by applying an HP in the TE of cartilage tissue. For instance, studying responses of monolayer cultures to HP, Suh et al. identified an increase of 40% in proteoglycan synthesis after applying 0.8 MPa alternating function times [47]. In addition, Jortikka et al. demonstrated that the GAG absorption raises due to a HP at 5 MPa and 0.5 Hz (dynamic compression) in contrast with static compression [48]. Even more, dynamic HP has proved that it also promotes a higher synthesis of aggrecan and type II collagen messenger RNAs (mRNAs) in mature chondrocytes (monolayer cultures) [49].

Concerning 3D matrices, the conclusions that can be drawn are slightly different in comparison with *in vivo* observations. Applying similar parameters to Jortikka's group, Parkkinen et al. showed a high increase in the incorporation of GAGs, but at much lesser rates [50]. Another interesting discovery obtained from 3D scaffolds are that adult cell lines and juvenile cell lines (chondrocytes) respond in diverse ways to mechanical stimuli (3–7 MPa at 0.25 Hz). Adult cells presented an increase in GAGs and type II collagen production, and juvenile cells only presented an increase in GAGs synthesis. This result implies that collagen synthesis is much more sensitive to HP than GAGs [51]. Even more, Elder et al. showed that static HP (5 or 10 MPa) was beneficial to scaffoldless explants to develop GAG and collagen synthesis, which consequently implied a higher compression stiffness of the generated ECM [52]. Thus, it can be argued that there are no clear responses and no accurate controls concerning the timing for HP application [40].

Also, HP has a differentiation role exercised on MSCs. A research group studied how the HP (0.1 MPa at 0.25 Hz) resulted in an increase in GAG and collagen concentrations in bone marrow MSCs (BMMSCs) in comparison with the control cases [53]. Other authors showed the raise of chondrogenesis markers Sox9, aggrecan and collagen II mRNAs in this model [54]. More interestingly, it has been demonstrated that HP leads to chondro-induction in other cell lines like fibroblasts, where the application of these forces lead to doubling of the production of GAG and collagen [55].

Together with HP, the delivery of TGFs, like TFG-β1 and TFG-β3, significantly helps the matrix construction in MSCs. For instance, TFG-β1 together with HP almost double ECM production together with its biomechanical properties (compressive and tensile stiffness) [56]. Other relevant pathways consequence of applying HP over chondrocytes is the down proliferation of pro-inflammatory signals, like IL-6, MMP-2 and MCP-1 [57].

4.3. Mechanical loads

Mechanical loads can be essentially explained as direct contacts between two surfaces. In AC the regular loads range from 0.5 to 8 MPa [58]. In the same manner that HP does, interstitial fluid supports the external compression *via* liquid pressurization [59]. This fact is responsible for increasing the stiffness of AC under dynamic loads [60]. Nevertheless, Armstrong et al. proved that interstitial fluid pressurization only supports the 33% of the compression load [61] and thus, the “solid” ECM supports the rest of the percentage of stresses.

Perhaps because of its simplicity and ease of use, the application of uniaxial stress over tissue surface is the most extended experimentation (Supplementary Table 1) of mechanobiology [62]. Using this technique it has been shown how physical stresses interact with the integrin receptors attached to the cell membrane activating G proteins and the adenosine 3′,5′-cyclic monophosphate (cAMP) signaling cascade [63]. Furthermore, it has been reported that the phospholipid membrane has the ability to activate G proteins under biomechanical stimulation by itself [64].

Mechanical stimuli directly interact with actin polymerization and depolymerization. Protein kinase A (PKA) phosphorylates Sox9 protein in adult chondrocytes, which enhances its transcriptional activity [65]. In addition, Yoon et al. showed how PKA regulates chondrogenesis in MSCs *via* a PKCα-dependent manner [66]. Juhász et al. determined that the chondrogenic response to compression regimes is related to elevated pSox9 levels. This result may be derived by the increase in the PKA enzyme activity from mechanically induced cartilage colonies. They also observed that Sox9 and cAMP response element-binding (CREB) expression and phosphorylation rise up after the application of mechanical stimulation [67]. External forces increase the quantity of Ser211 (a specific phosphorylated form of Sox9), which is directed involved in the ECM synthesis. Furthermore, Sox9 is activated by CREB due to physical interactions at the Ser133 [68]. Mechanical stimuli trigger the cAMP-PKA-dependent, the heterotrimeric Gαs-subunit, cAMP and the transcription factor CREB [69].

The mechanism behind how chondrocytes interact with external forces is still a hypothesis and is yet to be fully supported by experimental observations. Nevertheless, there is strong evidence that primary cilia, a sensory organelle acts as a link between the mechanical and chemical cues [70]. The primary cilium has also been observed in chondrocytes, MSCs and arthritic chondroprogenitor cells (CPCs) where it is used as a mechanosensor [71]. These cilia from chondrocytes are

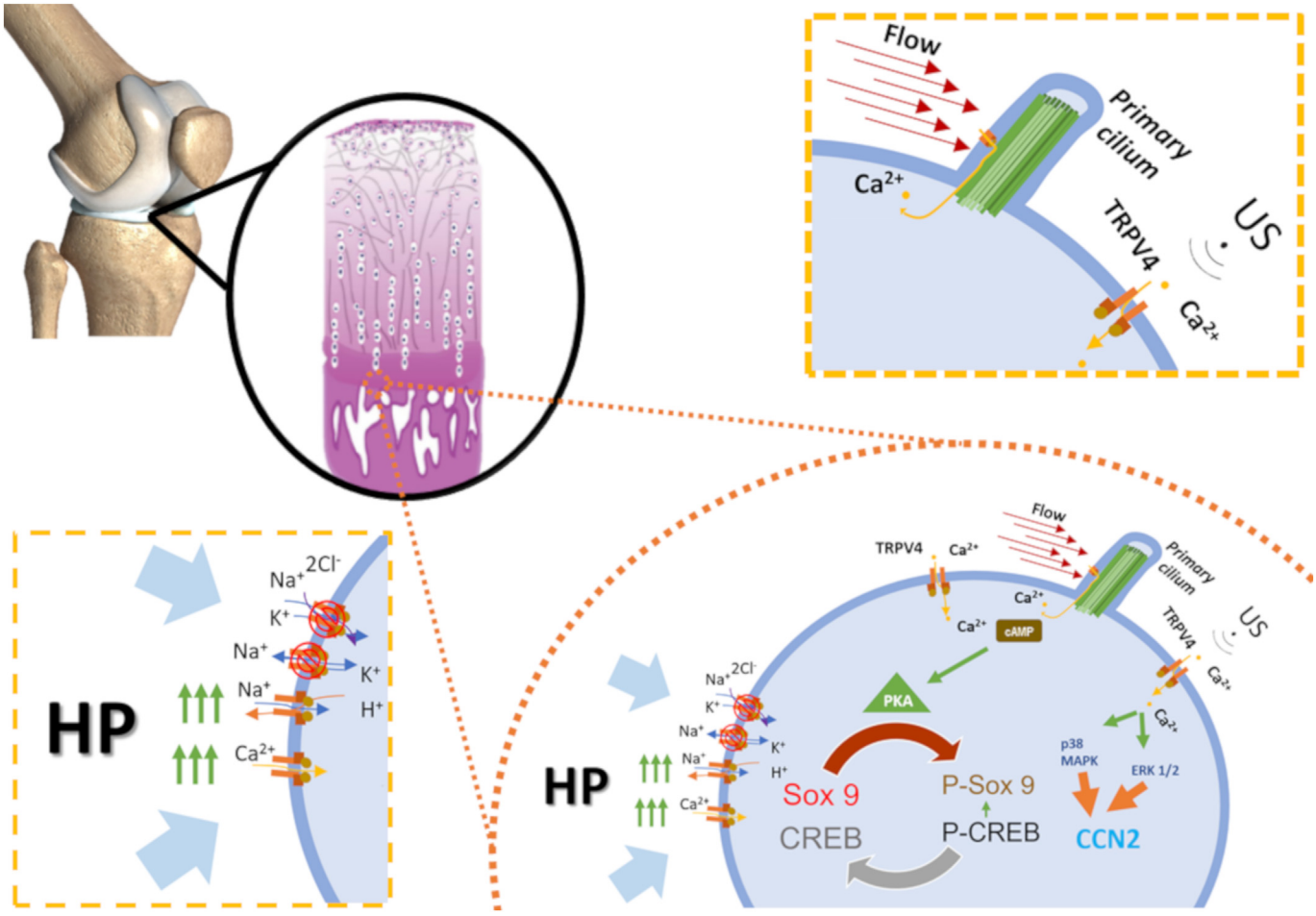

Fig. 3. Schematic representation of the biomechanical pathways involved in cartilage synthesis. Hydrostatic Pressure (HP) enhances Ca^{2+} and Na^{+} channels and the Na^{+}/K^{+} pump which interacts with PKA that regulates chondrogenesis (Sox9/CREB). In addition, Na^{+}/H^{+} pump activity is increased by HP. Both phenomena finally increase the intracellular concentration of Ca^{2+}. Shear stresses, induced by flow, stimulate the primary cilium of chondrocytes in a process called intraflagellar transport (IFT) which regulates pathways associated with type II & type IV collagen, Ca^{2+} channels which induces cAMP that phosphorylates the PKA. HP also plays a key role in the Sox9/CREB cycle. Finally, ultrasound (US) stimulation activates the TRPV4 and BK_{Ca} channels that activate MAPKs to induce CCN2 (chondrogenic factor). Even more LIPUS stimulates through actin polymerization the CCN2 molecule.

aligned with the ECM to respond to external forces. Wann et al. stimulated mutated chondrocytes (without cilia formation) and they found these chondrocytes were unsensitive to loading stimuli [72]. In addition, Farnum et al. demonstrated a difference in orientation of chondrocytes cilia between load-bearing cartilage and non-load-bearing one [73]. In contrast with other cells with cilia, the primary cilium in chondrocytes is essential for modulating the downstream process, which is called intra-flagellar transport (IFT), but not for early mechanoreception. Among these regulatory pathways are the ones associated with type II and IV collagen, G proteins, Indian hedgehog homolog (Ihh), and Ca^{2+} channels, connexins, purine, cAMP and the PKA pathway [74]. For instance, in the case of Ca^{2+} channels (Fig. 3) as polymodal transient receptor potential vanilloid (TRPV)-4, which is present in the chondrocyte cilia [75], were found to be induced by mechanical loading in porcine articular chondrocytes [76]. Another important example of membrane receptor of the cilia, is the case of connexin 43, a mechanosensitive ATP-release channel [77]. Thanks to these previous discoveries, it is possible that mechanical stimulation activates a signal transduction of the focal adhesion complexes (*e.g.*, integrins) activating adenylate cyclase to promote PKA by cAMP [67,78]. Also interesting is the finding that OA chondrocytes and healthy chondrocytes have differences in the length of cilia, meaning that this may act as a new biomechanical marker. In addition to this, it was proven that the length of cilia depends on IL 1β [74]. Rich et al. also found how chondrocyte cilia respond within minutes to changes in osmolarity, which implies an adjustment in cilia length [79].

The role of the interstitial fluid in AC is so critical that it is considered a biomarker for OA [80]. Interstitial fluid acts as low mechanical shear stresses over the chondrocytes producing stimulations previously discussed [59]. To mimic this natural *in vitro* interaction, perfusion flows (PFs) are applied in cartilage TE. Pazzano et al. demonstrated that after the application of a PF the chondrocytes were aligned in the same direction of the flow, resulting in an advantageous method for cartilage TE [81]. In previous studies, the use of PF has shown to be a strong tool to grow both cartilage and bone TE [82]. Nevertheless, recent literature remarks the importance of tailoring the flow velocity. Low flow velocity (10 μm/s and 7 μm/s) is appropriated for the early stages of the process in order to protect matrix deposition in the porous scaffolds and the type II collagen and GAGs synthesis [83]. After that, it is advisable to develop a slow increase in velocity (from 7 to 19 μm/s) [84].

Perfusion systems are also beneficial for treating OA. It has been found that PF decreases the hypertrophic regime of the pathological ECM. The synthesis of type I collagen under this bioengineered protocol is reduced, even more, adult arthritic chondrocytes displayed initial steps of redifferentiation [85].

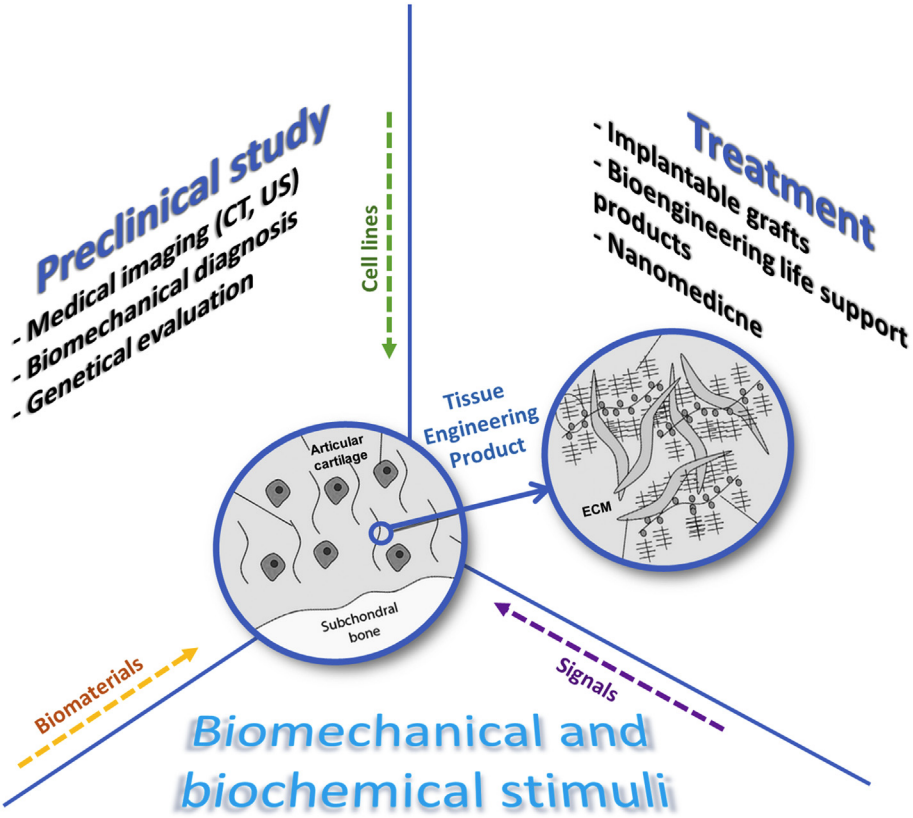

Fig. 4. Scheme of basis of future Biomedical Engineering for completed regeneration of damaged AC. The three axis of this scheme represents the pillars of tissue engineering: cell lines, biomaterials and stimuli. Finally, an accurate regenerative treatment can be done if it is added a previous biomechanical diagnosis and stimulation, which will improve the final advance regenerative therapy of OA.

4.4. Ultrasounds

Low-intensity pulsed ultrasound (LIPUS) is an acoustic wave that produces mechanical stimuli over cells and they approved by FDA for clinical therapy and bone fracture healing [86]. The applicability of LIPUS ($< 1\ W/cm^2$) for bone fracture healing has been demonstrated for several years [87]. LIPUS enhances calcium deposition and the synthesis of the bone morphogenic protein 2 (BMP-2) [88]. Moreover, LIPUS is also beneficial for fibroblasts, osteoblasts and chondrocytes proliferation both *in vitro* and *in vivo* [89]. Furthermore, there is evidence that LIPUS promotes the gene expression of type II collagen [90] and improves the synthesis of chondrogenic ECM [91].

Revising previous literature about LIPUS cell responses, there are several mechanotransduction routes involved in chondrocytes: the integrin/P13K/AKT pathway [92], the integrin-mediated p38 MAPK pathway [93], and the integrin-FAK/Src/p130Cas/CrkII/Erk pathway [94].

Recently, Nishida et al. found a positive feedback pathway which implied MAPK and the CCN family member 2, also known as connective tissue growth factor (CCN2) [95]. This growth factor is modulated by alterations in the cytoskeleton of fibroblasts and osteoblasts [96]. Specifically, it is known that CCN2 is expressed *via* actin polymerization. The same researchers have proven how CCN2 interacts with β- and γ- actin *in vitro* in human chondrosarcoma-derived chondrocytic cell line (HCS) [96]. In addition to the previous metabolic path, MAPK signaling is involved in the CCN2 induction under LIPUS stimulation; LIPUS promotes the Ca^{2+} influx through TRPV4 (Fig. 3), an BK_{ca} channel that activates MAPKs to induce CCN2 synthesis. Consequently, LIPUS stimulates in two different ways the CCN2 molecule, through actin polymerization and through MAPKs. which in turn results in an increase of chondrogenesis (Col2a and Acan over-expression).

LIPUS has also shown therapeutic results on OA, inhibiting protein expression of collagen type I and bone sialoprotein, as well as the gene expression of hypertrophic Col X [97]. In addition, LIPUS suppresses IL-1β (which implies MMP13 and ADAMTS 5, OA markers) as well as it helps to chondrocyte migration, proliferation and differentiation [86].

In conclusion, during the last years there have been many researchers that showed the great benefits of biomechanics understanding (Fig. 3) to treat AC disorders (*i.e.* OA). A summary of them are represented in Supplementary Table 1.

5. Future perspectives

Thanks to all the previous investigations done in the field of cartilage TE, it is not uncommon to find many diagnostic or analytical devices used in the clinic to clarify the biomechanics of the whole musculoskeletal system before applying any treatment or just for avoiding possible lesions. Namely, Auckland Bioengineering Institute (2014) developed an open software platform to cover all mechanisms behind the biomechanical behavior of the human body [98]. Furthermore, in the future it would be interesting to modulate the biogenesis of neocartilage from the biomechanical point of view not only to control the tissue development but also to tailor it for any application. The possibility to reproduce these biomechanical patterns in an *ex vivo* model for cartilage TE is also an interesting target. The use of bioreactors as a medical tool for tissue formation is highly recommended, not only for studying the cartilage development but also as therapeutic devices to develop advanced therapy medical products (ATMPs) for OA treatment (Fig. 4), for reducing inflammation and promoting ECM synthesis in the implanted area [99].

6. Conclusions

In this review, much of the evidence behind the physical arrangement and mechanical conformation of articular cartilage have been presented, which from the biomechanical point of view is not as simple as from the histological one. The interaction between the ECM and cells is essential not only for the correct working of the tissue, but also for

preventing disease, as could be OA. For these reasons, a more comprehensive estimation of the biomechanical pathway involved must be established to: i) detect disease at its initial stages ii) prevent the development of the disease, and iii) promote a real replacement of the AC using regenerative medicine techniques. Thus, the use of biomechanics as a medical tool for tissue formation is recommended as it has been previously studied in literature, not only for studying cartilage development but as a guide for building therapeutic devices for OA treatment, reducing inflammation and promoting ECM synthesis. In conclusion, biomechanics must be considered as an essential factor in cartilage formation and pathology; thereby, in regenerative medicine they need the same treatment as biochemical interactions.

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Transparency document

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Conflict of interest

None of the authors have a conflict of interest to declare.

Author contributions

All authors declare equally substantial contribution to de elaboration of this review in each section: conception and design of data acquisition, the article draft and the final approval version.

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