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Chapter

Epigenetics and Cartilage Regeneration

Samina Hyder Haq, Iqraa Haq, Atheer Ali Alsayah, Abir Alamro and Amani AlGhamedi

Abstract

Regenerative cartilage therapy has great potential for the treatment of debilitating diseases such as osteoarthritis and rheumatoid arthritis. Recent advances in the field of epigenetics have enabled us to understand more clearly the role of micro RNAs, DNA methylations and histone modification in disease progression, as well as its potential role in disease prevention. However, a thorough understanding of the external dietary and environmental factors that could affect the epigenetic events could be the key to unravelling novel therapeutic strategies for these diseases. There is, therefore, a need for identifying certain dietary or environmental factors that could change this downward epigenetics signalling cascade, stop or retard cartilage degradation and promote cartilage regeneration.

Keywords: cartilage regeneration, DNA methylations, epigenetics, therapeutic dietary supplements, DNMT inhibitors

1. Introduction

Articular cartilage is an aneural, avascular, alymphatic specialized fibrous connective tissue which covers the articulating surface of synovial joints. This is characterized by a small number of morphologically distinct populations of chondrocytes, which are primarily responsible for production, organization and maintaining the extensive network of an extracellular matrix. The balance between the hydration of matrix proteoglycans (PGs) and the resistance offered by the extensive network of the fibrous structure of the collagen provides the hydrodynamic load-bearing properties of articular cartilage, which is critical for joint movements and smooth transmission of mechanical compression across the joint. As articular cartilage is originally derived from the hyaline cartilage template, it is also classified as permanent hyaline cartilage. After the original phase of cartilage production, differentiation and resorption and closure of growth plate cartilage at puberty, it remains as a part of bone throughout the adult life. It is divided into four distinct horizontal layers: the superficial, transitional, deep and calcified cartilage layers (**Figure 1**).

The thin superficial zone protects the deeper layers from shear stress and injury and makes up 10–20% of articular cartilage thickness. This layer is characterized by small flattened disc-shaped chondrocytes, comparatively low proteoglycan content and densely packed layers of uniformly formed collagen fibres, which gives the characteristic hyaline opacity to cartilage. This layer is in direct contact to synovial fluids and is responsible for most of the tensile strength of the cartilage as well as

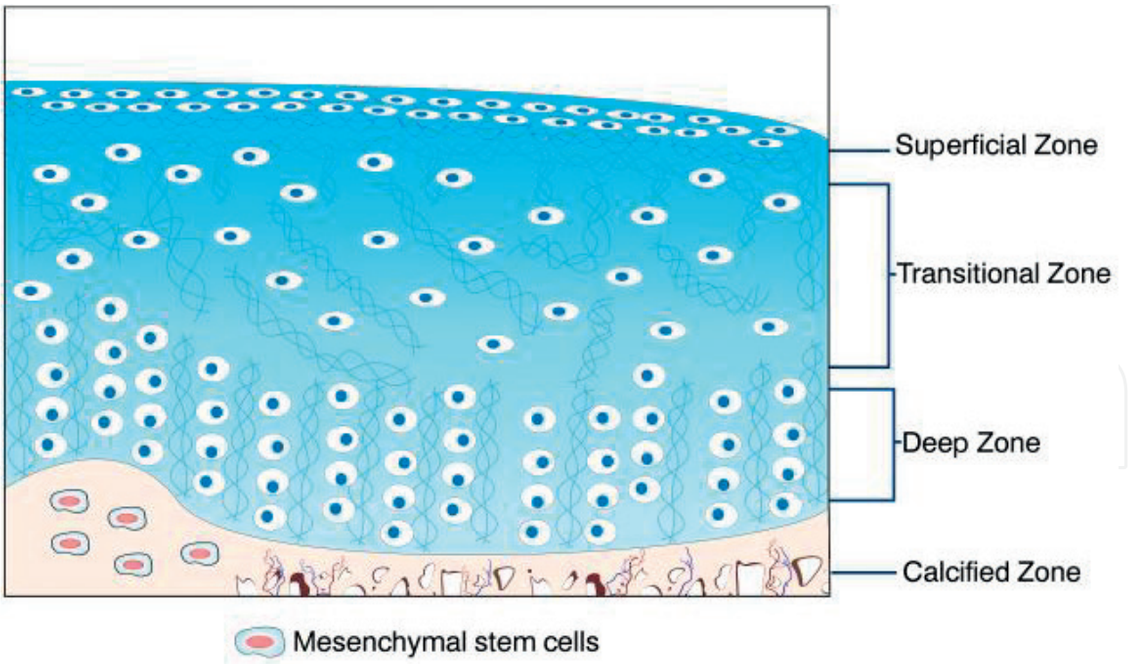


Figure 1.
Stratification of articular cartilage.

takes the direct brunt of inflammatory cytokines. It is well documented that the chronic inflammation in joints in osteoarthritis (OA) patients is due to synovial macrophages and high inflammatory cytokines that initiate the aggregenase, MMPs and other destructive enzymes. Immediately below the superficial zone is the middle or transitional layer which provides the functional bridge between the superficial and deep layers. The middle layer comprises of 40–60% of the total cartilage volume. In this layer, the chondrocytes attain a more rounded or spherical shape, the contents of proteoglycans increase, and thicker collagen fibres provide an oblique transitional network intermediate between the tangential superficial and radial deep layers. The deep layer is characterized by relatively mature rounded chondrocytes arranged in longitudinal columns, high proteoglycan contents, the largest diameter collagen fibrils in a radial disposition and the lowest water concentration. This zone represents approximately 30% of the total cartilage volume. The calcified layer is characterized by rounded hypertrophic chondrocytes surrounded by large clear lacunae. This is the area where the chondrocytes reach their terminal hypertrophic stage and the cartilage is ultimately being replaced by bone.

2. Molecular heterogeneity of articular cartilage

The extracellular phase of cartilage, and all connective tissues, consists of collagen fibres and a polysaccharide-rich ground substance. The polysaccharide constituents have been characterized as proteoglycans containing chains of chondroitin 4 sulphate, chondroitin 6 sulphate and keratin sulphate covalently linked to a central core protein [1].

2.1 Types of collagen present in cartilage

Articular cartilage consists of type II collagen as the major fibril-forming collagen, accompanied by lesser quantities of minor collagen which provide the tensile strength and help in maintaining the fine balance of the extracellular matrix. However, little is known about the processing of these minor collagens and their

role in the progression of cartilage degeneration and regeneration. Minor collagens found in articular cartilage along with type II collagen are type VI, IX, X, XI, XII and XIV.

Type VI collagen constitutes only 1–2% of the total collagen in adult articular cartilage and it is mainly rich in the pere-cellular matrix and involved in the integration and attachment of chondrocytes [2]. In articular cartilage, chondrocytes in the middle and deep layers are embedded in pere-cellular matrix enriched with a high content of proteoglycans and hyaluronic acid. Increased levels of type VI collagen are found in the experimental model of osteoarthritis (OA) and human OA [3]. Higher levels of type VI collagen found in OA emphasizes its role as a bridge between the extracellular matrix and the chondrocyte surface, thus influencing the signalling pathways from the extracellular matrix into the cells [4].

Type IX collagen makes up 1–5% of the total collagen in adult articular cartilage and 10% in foetal cartilage [5]. It is usually present in close association with type II collagen found in growth plate cartilage and adult articular cartilage [6]. Type IX collagen is extensively crosslinked to type II collagen through oxidation of lysyl residue bonds forming a unique hetero-fibrillar structure [7]. Type IX collagen is crucial for the maintenance of cartilage matrix and formation of a collagen fibril meshwork. Decreased expression of type IX collagen in the cartilage was thought to render the matrix more prone to mechanical forces and degradation, resulting in the pathogenesis of OA [8].

Type X constitutes about 1% of the total collagen found in articular cartilage [9]. It was revealed that 45% of the total collagen produced by the hypertrophic chondrocytes is type X collagen [10]. Type X collagen, as produced exclusively by hypertrophic chondrocytes, indicated its unique role in mineralization. The hypertrophic chondrocytes synthesized a variety of proteins and enzymes which help in the transition of extracellular matrix from cartilage to bone. Apart from type X collagen, hypertrophic chondrocytes also synthesize a variety of matrix metalloproteinases as well as alkaline phosphatase enzymes, which are not usually secreted by the normal proliferating chondrocytes. As type X collagen has a direct role in mineralization, it has been found to be expressed in human OA especially in the vicinity of lesions, but not in the healthy human articular cartilage [11].

Type XI collagen constitutes 3–10 % the total adult articular and foetal cartilage, respectively [2]. Type XI collagen is normally crosslinked to each other in cartilage, this crosslinking results in the formation of mature type XI collagen with the help of type II and type IX collagen. It has been shown that a mutation in type XI collagen caused an increase in degradation of type II collagen in articular cartilage [12]. Lu et al. observed that immunization of rats with homologous type XI collagen led to chronic and relapsing arthritis with different genetics and joint pathology than arthritis induced with homologous type II collagen [12]. The role of type XI collagen in cartilage collagen fibril formation and assembly is not clear; type XI collagen may regulate cartilage formation and it was the first collagen deposited by mesenchymal stem cells undergoing chondrogenic differentiation [13]. Type XII shares structural homologies with type IX and type XIV collagen [14]. Type XII collagen is implicated in fibril formation, cell adhesion, fibrosis and osteogenesis, and in areas of high mechanical stress, it may serve as a protector of tissue integrity [15]. Type XII collagen is associated with articular cartilage and growth plate cartilage during rat forelimb development and may be important for microenvironment that supports the hyaline cartilage formation [16].

Type XIV collagen is a large nonfibrillar extracellular matrix protein structurally similar to type XII collagen. In cartilage, a population of type XIV exists as chondroitin sulphate proteoglycans (PGs) as it is sensitive to chondroitinase ABC and AC treatments [17]. Its association with other cartilage collagens such as type I, II,

V and VI are reported, but it also interacts with heparin CD44 and cartilage oligomeric matrix protein [18]. It is found in areas of high mechanical stress similar to type XII collagen, suggesting its role in fibrillogenesis and maintaining the integrity and mechanical property of the tissue.

2.2 Types of PGs in different layers

Proteoglycans have the highest concentrations in the intermediate zone and lowest in the superficial and deep zones. Small PGs comprise of less than 10% of the total PG content in the cartilage matrix. Most are aggrecans (large PGs) with approximately 150 GAG chains (chondroitin sulphate and keratin sulphate and both O-linked and N-linked oligosaccharides attached).The GAGs are heterogeneously distributed along the protein core, with CS-rich and KS-rich regions, respectively. The protein core itself is heterogeneous with three globular regions. Aggrecan varies significantly in length, molecular weight and composition with the amount of KS-rich molecules and ratios of chondroitin 6-sulphate and chondroitin 4-sulphate increasing throughout development and ageing. Most aggrecans in cartilage are attached to a hyaluronic (HA) molecule via a globular (HABR) region; this binding was stabilized by a link protein. Several hundred aggrecans are attached to a single HA core molecule, the latter being a non-sulphated disaccharide chain up to 4 μm in length. PGs are closely associated with collagen fibrils and are thought to be involved in their structural organization and maintaining their compressive stiffness.

There is now conclusive evidence of the fact that OA is not simply due to wear and tear and a result of ageing; but in numerous studies, it has been reported that early onset of OA is due to activation of inflammatory response. These inflammatory responses could be due to increased oxidative stress to the tissues, resulting in initiation of catabolic enzymes and factor that actively breakdown the major extracellular matrix components of cartilage, namely type II collagen, and the proteoglycans and aggrecan.

3. Control of chondrogenesis

The commitment of mesenchymal cells to the chondrogenic lineage is the key event in bone formation. Work over the past few decades, using both in vivo and in vitro systems, has identified a number of signalling and transcription factors as well as cell shape that regulates the progressive change in chondrocyte phenotype, from their initial induction to their terminal fate. The disruption of these finely tuned pathways for chondrocyte maturation can result in skeletal pathology. A thorough knowledge of these signalling pathways would help us to identify the factors that maintain chondrocyte proliferation and differentiation. Some of the major signalling pathways are described below.

3.1 Bone morphogenic protein signalling

Bone morphogenic proteins (BMPs) are identified as positive regulators of chondrogenesis and endochondral ossification. BMPs are a member of the transforming growth factor beta (TGβ) superfamily that has wide-ranging biological activity, ranging from cellular regulation of proliferation, apoptosis, differentiation and migration [19, 20]. BMP signalling is mediated by their receptors BMPR1a, BMPR1b and BMPR2, leading to the SMAD signalling pathway [19]. In cartilage, it initiates cartilage synthesis and decreases the activity of catabolic cytokines such as IL-1, IL-6, IL-8, MM1 and MM13 [21, 22]. Though there are several members of

Bone morphogenic protein (BMP) growth factors, most promising among them in the treatment of OA is BMP-7, which promotes the cartilage-specific extracellular matrix proteins such as collagen II and VI, decorin, fibronectin and hyaluronate (HA) by upregulation of hyaluronan synthase [23, 24]. In experiments when it was applied to other types of cells in knee, BMP-7 has shown to increase Extracellular matrix (ECM) in synovial and bone marrow-derived Mesenchymal Stem cell (MSC), both alone and in combination with TGFβ [25]. This profound anabolic effect of BMP-7 is due to its regulatory properties of modulating other growth factors such as insulin-like growth factor 1(ILGF1 and fibroblast growth factor (FGF)) [26]. Despite having anabolic activity, BMP-7 has not shown to induce chondrocyte hypertrophy or other changes in the chondrocyte phenotype, nor did BMP-7-treated animal knee display any proliferation of fibroblast or osteocyte [25]. These properties make it a promising therapy for OA.

3.2 Transforming growth factor (TGF) signalling pathway

TGFβ is a cytokine secreted by many cells; it plays an important role in cell proliferation, differentiation, development, apoptosis, tissue homeostasis and the immune system. Signalling occurs through SMAD pathways. TGβ1 is shown to be involved in chondrocyte proliferation and remarkable reduction of catabolic activity of IL1 and TNF [27]. Studies have shown a significant enhancement of cartilage repair with the application of TGF-β1 in scaffold applied to defect, and in human MSC transfected with TGF-β1 gene via an adenovirus [28, 29]. Numerous human trials are underway for the treatment of different stages of OA with the injections of TGF-β1 in the knee, which showed TGF-β1 as a promising therapy.

3.3 Fibroblast growth factor signalling pathway

Fibroblast growth factor (FGF) family plays an important role in human embryonic development, cell growth, morphogenesis, tissue repair, tumour growth and invasion. FGFs are heparin-binding proteins and interact with heparan sulphate proteoglycans on the cell surface for signal transduction. Vincent et al. proposed that in articular cartilage, the chondrocytes are surrounded by a pool of FGF-2. This mediated the chondrocyte activation on cartilage loading and release of FGF-2 in response to injury. They proposed that FGF-2 antagonizes the PG degradation by IL-1 or other catabolic stimuli, thus it has an anti-catabolic chondroprotective role [30]. However, the role of FGF-2 in the production of ECM is controversial and its role as pro-catabolic or anti-catabolic is debatable. Furthermore, FGF-2 has been shown to suppress type II collagen and PG synthesis and promote the expression of aggregenase and TNF-α receptors [31, 32]. FGF-18 signalling through FGFR3 promotes chondrocyte proliferation at embryonic stages. When development is complete, the same receptor signalling suppresses chondrocyte proliferation and prevents chondrocyte differentiation hypertrophy [33, 34]. FGF-18 has also shown to exhibit the ability to stimulate type II collagen and PG synthesis, which makes it a promising therapy for OA.

3.4 Connective tissue growth factor

Connective tissue growth factor (CTGF) is an ECM-associated heparin-binding protein, which plays an important role in cellular proliferation, migration, adhesion, survival and synthesis of ECM proteins. CTGF has shown to play an important role in skeletal tissue and initial chondrocyte proliferation and differentiation in growth plate cartilage [35]. Nishida and colleagues demonstrated that local

administration of recombinant CTGF gelatin hydrogel stimulated cartilage repair in rat model [36]. Other studies showed that the bone marrow-derived mesenchymal stem cells when transfected with CTGF provided hyaline-like cartilage regeneration similar to normal cartilage in a rabbit model of focal articular cartilage defects [37]. However, further studies are needed to elucidate the critical role of CTGF for the protection and regeneration of cartilage.

3.5 Insulin-like growth factor (IGF)

IGF-I and IGF-II both were reported to control the cartilage destruction [38]. IGF-I is a known anabolic factor for chondrocytes and thought to regulate the skeletal development in the embryo [39]. Although IGF-I has been reported as being involved in chondrocyte proliferation and maturation, its exact role in OA has not been clearly known as it was found that the expression of IGF-I was upregulated rather than downregulated in synovial fluids and in articular cartilage [40]. However, the role of IGF-II in combating inflammatory response in OA was found to be more promising and ideal for cartilage regeneration. It has been reported that in the presence of IL-1β, IGF-II significantly inhibited MMP expression and promoted cartilage production in normal human chondrocytes. IGF-II has also shown to have a similar effect on OA chondrocyte, which expresses high levels of IL-1β mRNA [41]. The role of IGF-II was reported to be more chondroprotective and maintaining the extracellular matrix and preventing its destruction in adverse conditions.

4. Cell signalling in chondrogenesis

Gene expression changes during different stages of endochondral ossification. The immature chondrocytes in the resting zone express the transcription factors Sox 5, Sox6, Sox9 and the structural protein type II collagen and aggrecan. The pre-hypertrophic zone is characterized by the presence of parathyroid receptor 1(PTH-1R) and Indian hedgehog expression (Ihh). The next stage goes into the early hypertrophic zone, which is characterized by type X collagen and alkaline phosphatase enzyme expression, and, subsequently, the reduced amount of type II collagen and reduced expression of Sox5, Sox6 and Sox9 transcription factors. Finally, the chondrocytes proceed to their final phase of a late hypertrophic stage, which is characterized by the expression of vascular endothelial growth factor A (VEGFA), matrix metalloproteinase 13(MM13) and osteopontin. These changes in gene expression herald the cartilage ECM being replaced by bone.

Wnt signalling is important for many developmental processes. It has been shown that activation of Wnt signalling promotes osteoblast differentiation but inhibits chondrocyte differentiation of MSC [42, 43]. Wnt signalling acts through β-catenin to promote chondrocyte hypertrophy and reports suggested that genetic inactivation of β-catenin increased Sox9 expression both in the intramembranous bone formation and endochondral ossification [44, 45]. It was also reported that osteoblast precursor lacking β-catenin expression can develop into chondrocytes [42]. Wnt signalling is also important for proper orientation of chondrocyte column in growth plate cartilage.

Ihh signalling is a key regulator of pre-hypertrophic and early hypertrophic chondrocytes. Ihh signalling directly affects chondrocyte proliferation, premature chondrocyte hypertrophy and failure of osteoblast development and endochondral bone.

Runx2 and Runx3 are members of the Runx transcriptional factors family important for chondrocyte hypertrophy. Several studies demonstrated that ectopic

expression of Runx2 in immature chondrocytes leads to the expression of hypertrophic markers such as COLX α1, MM13 and VEGF [46–48].

As cartilage is an avascular tissue and its nutritional needs are met by surrounding synovial fluids, chondrocytes are adapted to survive in low oxygen levels and they secrete hypoxia-inducible factor 1 (HIF-1) which insures its survival and maintenance in low oxygen tension. Synthesis of type II collagen and aggrecan is upregulated in low oxygen levels, and also, it is associated with the rounded chondrocytic morphology. In the presence of high oxygen tension, chondrocytes become more spindle shaped. HF-1 also showed inhibition of type I collagen synthesis by inhibiting its promoter activity [49].

5. Epigenetic control of chondrogenesis

In the growth of long bone formation, the chondrocyte passes through discrete stages of proliferation, maturation, hypertrophy, calcification and apoptosis, so it offers a very good model of cellular differentiation and ageing. The detailed underlying molecular mechanisms that drive these changes are still not fully known, but epigenetic modifications are thought to play a pivotal role in the differentiation of chondrocytes. Epigenetic changes include DNA methylation, histone modification and microRNAs (miRNAs).

5.1 DNA methylation

DNA methylation involves the addition of a methyl group to a DNA at CpG dinucleotide, to convert cytosine to 5-methylcytosine. CpG islands are usually clustered near the promoter in about 30% of the gene. Methylation of these sequences results in silencing of these genes, and vice versa, hypo-methylation results in expression of the respective genes. DNA methylation factors are established and modified according to the environmental factors by three DNA methyltransferases (DNMT1, DNMT3a and DNMT3b). Earlier studies using chick embryos indicate the possible role of methylation in gene expression of type I and type II collagen in chondrocyte differentiation and dedifferentiation [50]. In our studies on chick chondrocytes in culture, we noticed a strong correlation of chondrocyte morphology to DNA methylation status as shown in **Figure 2**. The chondrocytes when treated with DNMT inhibitor 5-aza-2'deoxycytidine exhibit fibroblastic morphology and express type I and type X collagen with an upregulation of alkaline phosphatase enzyme [51]. Two CpG sites within the type X collagen promoter appear to be demethylated during MSC differentiation into chondrocyte morphology [52]. Recently, it was demonstrated that Wnt signalling caused both repressive chromatin mark (H3K27me3) and DNA methylation over the SOX9 promoter and that Wnt-induced irreversible silencing of Sox9 gene requires DNA methylation of this locus that is specifically countered by FGF signalling [53]. FGF blocks the recruitment of DNMT3a to the SOX-9 promoter by inducing the interaction and phosphorylation of DNMT3a by extracellular kinases ERK 1and ERK 2. Similarly, a number of studies indicated the control of Runx2 promoter activation by methylation. The number of MMP promoters show decreased methylation at single CpG island in OA cartilage as compared to normal.

5.2 Histone modifications

Gene regulation is also controlled through the close packaging of eukaryotic DNA into nucleosomes. Nucleosomes are thought to be repressive for

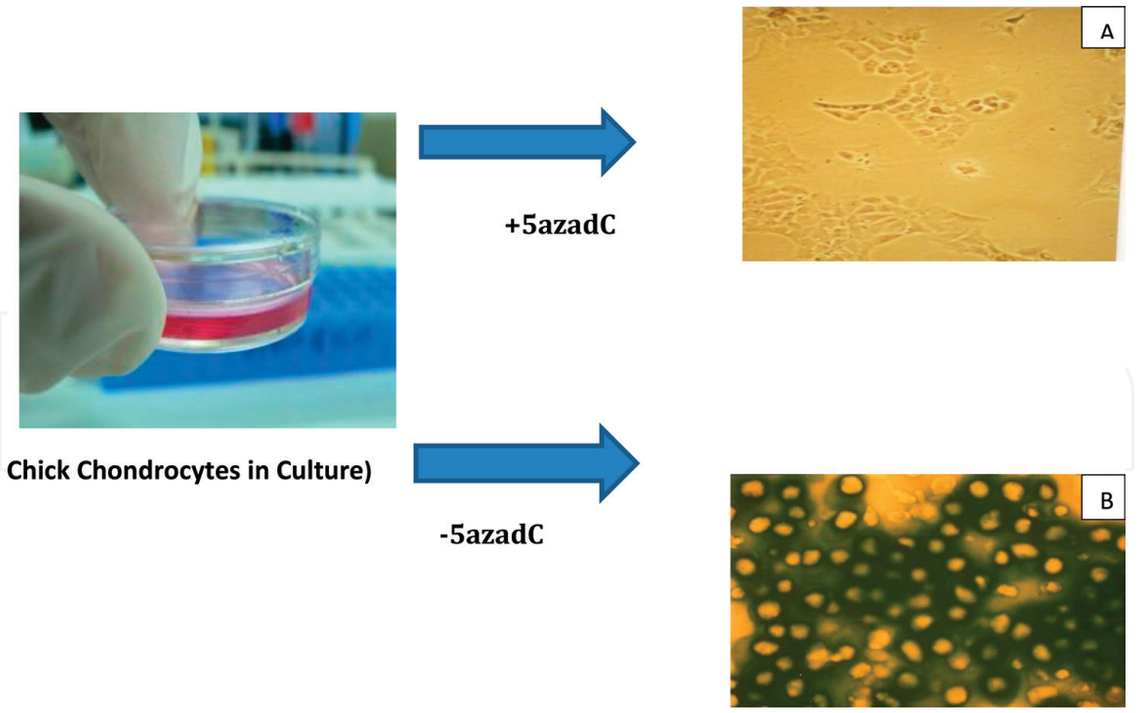


Figure 2.
The effect of culture conditions on the morphology of chondrocytes: When chick chondrocytes from caudal region sternum were grown in the presence of demethylation drug 5aza-2'deoxyctydine (5azadC), (A) the chondrocytes assume more flattened fibroblastic morphology and show no staining with alcian blue (stain specific for sulphate PG). However, the control chondrocyte without any treatment showed extensive ECM staining (B).

transcription; but through the post-translational modification of histones such as acetylation, phosphorylation, methylation and ubiquitination, this inhibition can be regulated.

Acetylation is mediated through acetyltransferase (HAT) and occurs on specific lysine residues on the N-terminal tails of histones, loosening the histones: DNA interactions, thus employing the access of transcriptional factors to the DNA. Deacetylation is of two types, one that requires Zn-catalysed deacetylation (HDAC) and the sirtuin deacetylase that requires NAD+, and removes these acetyl groups resulting in hypo-acetylation. Numerous transcriptional activators or repressors recruit HDAC and HAT activity.

Histone methylation is important for the formation of active and inactive genomic regions and is associated with transcription activation and silencing. Methylation of histone tails of lysine and arginine residues is catalysed by histone methyltransferase (HMT) and protein arginine methyltransferase (PRMT) which can add one or more methyl groups to regulate transcription [54]. Although histone methylation is more dynamic than DNA methylation, some specific histone methylation is tightly regulated and maintained through DNA replication. HDAC can block cytokine-induced PG release and cartilage resorption in cartilage explant model indicating that HDAC activity is important for the catabolic activity of chondrocytes [55, 56].

5.3 Micro RNA

MiRNA is a small 20–23 base pair-long cytoplasmic RNA that regulates post-transcriptional gene expression through binding to target mRNA. This

interaction of miRNA with the target mRNA results in degradation of mRNA, thus suppression of translation. The first studied miRNA in cartilage was miR-140, which was first identified as cartilage restricted in developing zebrafish [57]. In humans, the expression of miR-140 increases during chondrogenesis and is more abundant in articular cartilage, but its expression is reduced in OA [58]. It has also been reported that the expression of miR-140 is regulated by the cartilage-specific master transcriptional factor Sox-9 in zebrafish and mammalian cells [59].

6. Epigenetics as a future therapy for cartilage regeneration

Articular cartilage has a relatively high incidence of damage due to several factors such as injury, trauma and inflammation. The inflammatory markers could induce a number of MMPs, which could degrade the ECM, as the cartilage has a limited ability to repair and regenerate, resulting in a total loss of cartilage tissue. The destruction and loss of articular cartilage is also central to the development of OA. The research work over the past few decades confirms that epigenetics plays a pivotal role in the phenotypic modulation that articular chondrocytes undergo during OA. Epigenetics changes the normal chondrocytes to 'altered' chondrocytes that overexpress the cartilage-degrading proteins or enzymes such as collagenases and aggregenase and inflammatory mediators. This disruption in homeostatic balance between the matrix production and ECM destruction results in the progression of OA. There is a direct pathological loop that involves inflammation and epigenetic modifications, which accelerates disease progression. Until now, no detailed global methylation analysis has been performed in the pathogenesis of OA. Low penetrance polymorphism in the population partly due to epigenetic modification is the reason for limited data generation to aid in the identification of genes responsible for the genetic susceptibility to OA. A number of inflammatory genes have been identified which are controlled through epigenetics and are directly involved in the pathogenesis of OA (**Table 1**).

6.1 Future prospects in cartilage regeneration

MCS is becoming a more popular source of cells for cartilage regeneration due to in vitro expansion without running the risk of losing their phenotype. However, MSC tends to develop hypertrophic phenotype and further differentiation into the endochondral bone formation. It is becoming more crucial to carefully examine the detailed molecular and epigenetic events that lead the transformation of a chondrocyte to its terminally differentiated pathway. There is a growing need to develop strategies to control chondrocyte hypertrophy and be able to arrest the chondrocyte at one desirable phenotypic stage that helps to maintain the cartilage-specific ECM as described in **Figure 3**. With the current epigenetic knowledge, it is possible to identify a number of epigenetic factors as listed in **Table 1** that can make cartilage regeneration possible.

Other option in cartilage regeneration is the application of hydrogel through injection or through arthroscopy. These hydrogels are capable of controlled release of chondroinductive and chondroprotective drugs [60–62]. These cell-laden hydrogels can be combined with other types of solid scaffolds such as collagen sponge, decellularized cartilage as well as synthetic scaffolds of polyglycolic acid for cartilage repair and clinical applications.

Chondrocyte stage	Marker	Function gene	Epigenetic regulation	References
Superficial zone	Col2a1	Cartilage specific	miRNA, Histone modification	[1]
	Col6a1	Pere-cellular chondrocyte	DNA methylation	[2]
	Col9a1	Cartilage specific	DNA methylation	[3]
	ACAN	Cartilage specific	miRNA, Histone modification	[4, 5]
	HIF1α, HIF2α	Chondrocyte viability	miRNA	[6]
Transitional	IGFII	Chondrocyte proliferation and integrity		[7]
	SOX-9	Chondrocyte differentiation	DNA methylation, miRNA, histone methylation	
	T3 +PTH	Cartilage tissue regeneration	Histone modification	[8, 9]
	NFAT	Cartilage matrix	Histone methylation	[10]
	FGFR3	Chondrogenesis	DNA methylation	[10]
	TGFβ1-β3	Chondrocyte proliferation	DNA methylation	[11, 12]
	BMP-7	Cartilage specific ECM	Histone modification, DNA methylation	[11, 13]
Deep/ Calcifying	Col10a1	Chondrocyte hypertrophy	DNA methylation	[14]
	Col1α1	Bone formation	DNA methylation	[15]
	Osteocalcin	In calcification	DNA methylation	[16]
	Osteopontin	Bone formation	DNA methylation	[16]
	ALPL	Chondrocyte hypertrophy	DNA methylation	[17]
	RUNX 2	Chondrocyte hypertrophy	DNA methylation, miRNA	[18, 19]
	ADAM	Cartilage remodelling	DNA methylation, miRNA	[10]
	IHH	Cartilage hypertrophy	DNA methylation	[10]
	TGFβ2	Hypertrophy	DNA methylation	[20, 21]
	MMP13	Cartilage remodelling	DNA methylation, histone modification, miRNA	[1]
OA cartilage	HDAC	Up regulated in OA	Histone modification	[22]
	IL-1β	Inflammation	DNA methylation, miRNA	[23]
	TNFα	Inflammatory mediator	DNA methylation, miRNA, histone modification	[11]
	MMP3	Up regulated in OA	DNA methylation	[24]
	MMP9	Up regulated in OA	DNA methylation	[24]
	ADAMS4	Expressed in OA	DNA methylation	[24]

Table 1.
Major Epigenetic events remodelling the regeneration of Cartilage.

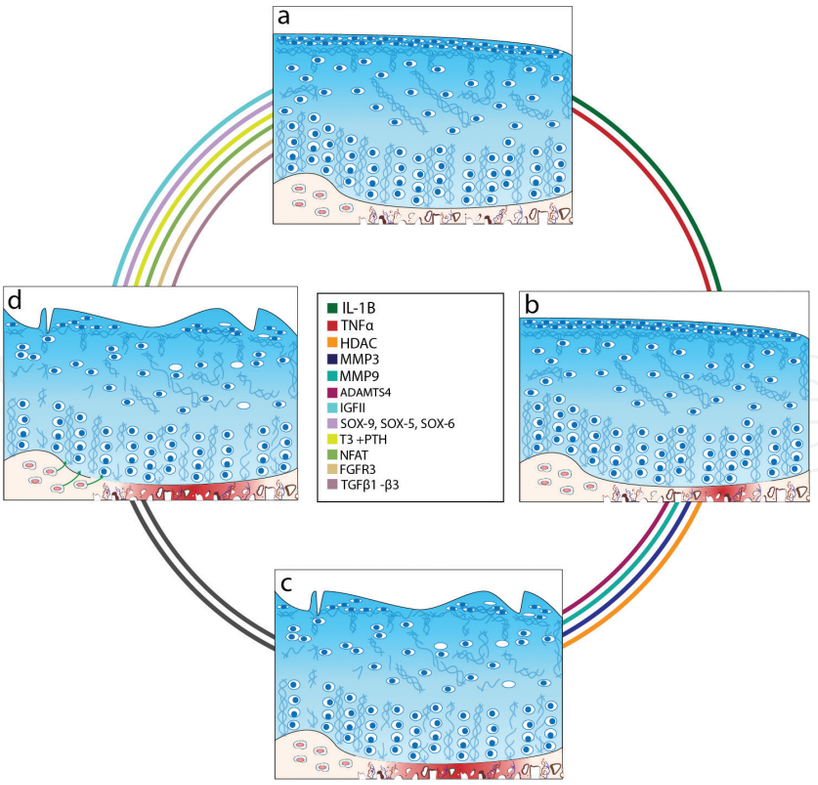


Figure 3.
*The role of epigenetics in cartilage degradation and regeneration. (a) Healthy articular cartilage with distinct stratifications. (b) As a result of high inflammatory markers such as IL-1β and TNFα, cartilage degradation takes place, with upregulation of a number of cartilage-degrading enzymes (e.g., HDAC, MMP3, MMP9, and ADAMTS4). (c) The onset of OA, which can be reversed with the help of MSC therapy and their initiations as shown in (d). The maintenance of healthy articular cartilage is achieved through a cascade of genes and their products, such as IGFII, SOX5, SOX6, SOX9, NFAT, FGFR3 and TGFβ1-β2. They are all controlled through epigenetics (***Table 1***). Future cartilage regeneration technique should involve the promotion of invasion and migration of MCSs to the lesion area and through various epigenetic signalling undergoing chondrogenesis and maintaining the cartilage.*

7. Conclusion

In summary, although there has been progress made in identifying factors outlining OA disease progression, a more detailed analysis of the factors surrounding the epigenetics should be conducted in order to reveal any potential therapies. The control of chondrogenesis via bone morphogenic protein signalling, transforming growth signalling, fibroblast growth factor signalling, connective tissue growth factor and insulin-like growth factor all play important roles in chondrocyte formation and destruction. This in addition to the fact that cellular mechanisms controlled by gene expression and epigenetic changes including DNA methylation, histone modification, and microRNAs can all help us gain an understanding of regenerative cartilage therapies.

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Conflict of interest

There is no conflict of interest to declare.

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References

[1] McDevitt CA. Biochemistry of articular cartilage. Nature of proteoglycans and collagen of articular cartilage and their role in ageing and in osteoarthrosis. Annals of the Rheumatic Diseases. 1973;**32**(4):364-378

[2] Eyre D. Collagen of articular cartilage. Arthritis Research. 2002;**4**(1):30-35

[3] Pullig O, Weseloh G, Swoboda B. Expression of type VI collagen in normal and osteoarthritic human cartilage. Osteoarthritis and Cartilage. 1999;**7**(2):191-202

[4] Horikawa O, Nakajima H, Kikuchi T, Ichimura S, Yamada H, Fujikawa K, et al. Distribution of type VI collagen in chondrocyte microenvironment: Study of chondrons isolated from human normal and degenerative articular cartilage and cultured chondrocytes. Journal of Orthopaedic Science. 2004;**9**(1):29-36

[5] Eyre DR, Weis MA, Wu JJ. Articular cartilage collagen: an irreplaceable framework? European Cells & Materials. 2006;**12**:57-63

[6] Eyre DR, Apon S, Wu JJ, Ericsson LH, Walsh KA. Collagen type IX: evidence for covalent linkages to type II collagen in cartilage. FEBS Letters. 1987;**220**(2):337-341

[7] Wu JJ, Woods PE, Eyre DR. Identification of cross-linking sites in bovine cartilage type IX collagen reveals an antiparallel type II-type IX molecular relationship and type IX to type IX bonding. The Journal of Biological Chemistry. 1992;**267**(32):23007-23014

[8] Alizadeh BZ, Njajou OT, Bijkerk C, Meulenbelt I, Wildt SC, Hofman A, et al. Evidence for a role of the genomic region of the gene encoding for the alpha1 chain of type IX collagen (COL9A1) in hip osteoarthritis: A population-based study. Arthritis and Rheumatism. 2005;**52**(5):1437-1442

[9] Eyre DR, Wu JJ, Woods PE, Weis MA. The cartilage collagens and joint degeneration. British Journal of Rheumatology. 1991; **30**(Suppl 1):10-15

[10] Shen G. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. Orthodontics & Craniofacial Research. 2005;**8**(1):11-17

[11] Brew CJ, Clegg PD, Boot-Handford RP, Andrew JG, Hardingham T. Gene expression in human chondrocytes in late osteoarthritis is changed in both fibrillated and intact cartilage without evidence of generalised chondrocyte hypertrophy. Annals of the Rheumatic Diseases. 2010;**69**(1):234-240

[12] Lu S, Carlsen S, Hansson AS, Holmdahl R. Immunization of rats with homologous type XI collagen leads to chronic and relapsing arthritis with different genetics and joint pathology than arthritis induced with homologous type II collagen. Journal of Autoimmunity. 2002;**18**(3):199-211

[13] Xu J, Wang W, Ludeman M, Cheng K, Hayami T, Lotz JC, et al. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue Engineering. Part A. 2008;**14**(5):667-680

[14] Yamagata M, Yamada KM, Yamada SS, Shinomura T, Tanaka H, Nishida Y, et al. The complete primary structure of type XII collagen shows a chimeric molecule with reiterated fibronectin type III motifs, von Willebrand factor A motifs, a domain homologous to a noncollagenous region of type IX collagen, and short

collagenous domains with an Arg-Gly-Asp site. The Journal of Cell Biology. 1991;**115**(1):209-221

[15] Chiquet M, Birk DE, Bonnemann CG, Koch M. Collagen XII: Protecting bone and muscle integrity by organizing collagen fibrils. The International Journal of Biochemistry & Cell Biology. 2014;**53**:51-54

[16] Taylor DW, Ahmed N, Parreno J, Lunstrum GP, Gross AE, Diamandis EP, et al. Collagen type XII and versican are present in the early stages of cartilage tissue formation by both redifferentating passaged and primary chondrocytes. Tissue Engineering. Part A. 2015;**21**(3-4):683-693

[17] Watt SL, Lunstrum GP, McDonough AM, Keene DR, Burgeson RE, Morris NP. Characterization of collagen types XII and XIV from fetal bovine cartilage. The Journal of Biological Chemistry. 1992;**267**(28):20093-20099

[18] Giry-Lozinguez C, Aubert-Foucher E, Penin F, Deleage G, Dublet B, van der Rest M. Identification and characterization of a heparin binding site within the NC1 domain of chicken collagen XIV. Matrix Biology. 1998;**17**(2):145-149

[19] Goumans MJ, Mummery C. Functional analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. The International Journal of Developmental Biology. 2000;**44**(3):253-265

[20] Massague J, Chen YG. Controlling TGF-beta signaling. Genes & Development. 2000;**14**(6):627-644

[21] Badlani N, Oshima Y, Healey R, Coutts R, Amiel D. Use of bone morphogenic protein-7 as a treatment for osteoarthritis. Clinical Orthopaedics and Related Research. 2009;**467**(12):3221-3229

[22] Elshaier AM, Hakimiyan AA, Rappoport L, Rueger DC, Chubinskaya S. Effect of interleukin-1beta on osteogenic protein 1-induced signaling in adult human articular chondrocytes. Arthritis and Rheumatism. 2009;**60**(1):143-154

[23] Chubinskaya S, Hurtig M, Rueger DC. OP-1/BMP-7 in cartilage repair. International Orthopaedics. 2007;**31**(6):773-781

[24] Chubinskaya S, Hakimiyan A, Pacione C, Yanke A, Rappoport L, Aigner T, et al. Synergistic effect of IGF-1 and OP-1 on matrix formation by normal and OA chondrocytes cultured in alginate beads. Osteoarthritis and Cartilage. 2007;**15**(4):421-430

[25] Shen B, Wei A, Whittaker S, Williams LA, Tao H, Ma DD, et al. The role of BMP-7 in chondrogenic and osteogenic differentiation of human bone marrow multipotent mesenchymal stromal cells in vitro. Journal of Cellular Biochemistry. 2010;**109**(2):406-416

[26] Chubinskaya S, Otten L, Soeder S, Borgia JA, Aigner T, Rueger DC, et al. Regulation of chondrocyte gene expression by osteogenic protein-1. Arthritis Research & Therapy. 2011;**13**(2):R55

[27] Lires-Dean M, Carames B, Cillero-Pastor B, Galdo F, Lopez-Armada MJ, Blanco FJ. Anti-apoptotic effect of transforming growth factor-beta1 on human articular chondrocytes: Role of protein phosphatase 2A. Osteoarthritis and Cartilage. 2008;**16**(11):1370-1378

[28] Abe T, Yamada H, Nakajima H, Kikuchi T, Takaishi H, Tadakuma T, et al. Repair of full-thickness cartilage defects using liposomal transforming growth factor-beta1. Journal of Orthopaedic Science. 2003;**8**(1):92-101

[29] Diao H, Wang J, Shen C, Xia S, Guo T, Dong L, et al. Improved cartilage

regeneration utilizing mesenchymal stem cells in TGF-beta1 gene-activated scaffolds. Tissue Engineering. Part A. 2009;**15**(9):2687-2698

[30] Vincent TL, Hermansson MA, Hansen UN, Amis AA, Saklatvala J. Basic fibroblast growth factor mediates transduction of mechanical signals when articular cartilage is loaded. Arthritis and Rheumatism. 2004;**50**(2):526-533

[31] Im HJ, Li X, Muddasani P, Kim GH, Davis F, Rangan J, et al. Basic fibroblast growth factor accelerates matrix degradation via a neuro-endocrine pathway in human adult articular chondrocytes. Journal of Cellular Physiology. 2008;**215**(2):452-463

[32] Ellman MB, Yan D, Ahmadinia K, Chen D, An HS, Im HJ. Fibroblast growth factor control of cartilage homeostasis. Journal of Cellular Biochemistry. 2013;**114**(4):735-742

[33] Liu Z, Xu J, Colvin JS, Ornitz DM. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. Genes & Development. 2002;**16**(7):859-869

[34] Liu Z, Lavine KJ, Hung IH, Ornitz DM. FGF18 is required for early chondrocyte proliferation, hypertrophy and vascular invasion of the growth plate. Developmental Biology. 2007;**302**(1):80-91

[35] Nakao K, Kubota S, Doi H, Eguchi T, Oka M, Fujisawa T, et al. Collaborative action of M-CSF and CTGF/CCN2 in articular chondrocytes: Possible regenerative roles in articular cartilage metabolism. Bone. 2005;**36**(5):884-892

[36] Hoshijima M, Hattori T, Aoyama E, Nishida T, Yamashiro T, Takigawa M. Roles of heterotypic CCN2/CTGF-CCN3/NOV and homotypic CCN2-CCN2 interactions in expression of the differentiated phenotype of chondrocytes. The FEBS Journal. 2012;**279**(19):3584-3597

[37] Zhu S, Zhang B, Man C, Ma Y, Liu X, Hu J. Combined effects of connective tissue growth factor-modified bone marrow-derived mesenchymal stem cells and NaOH-treated PLGA scaffolds on the repair of articular cartilage defect in rabbits. Cell Transplantation. 2014;**23**(6):715-727

[38] Mullen LM, Best SM, Ghose S, Wardale J, Rushton N, Cameron RE. Bioactive IGF-1 release from collagen-GAG scaffold to enhance cartilage repair in vitro. Journal of Materials Science. Materials in Medicine. 2015;**26**(1):5325

[39] Tahimic CG, Wang Y, Bikle DD. Anabolic effects of IGF-1 signaling on the skeleton. Frontiers in Endocrinology (Lausanne). 2013;**4**:6

[40] Matsumoto T, Gargosky SE, Iwasaki K, Rosenfeld RG. Identification and characterization of insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and IGFBP proteases in human synovial fluid. The Journal of Clinical Endocrinology and Metabolism. 1996;**81**(1):150-155

[41] Uchimura T, Foote AT, Smith EL, Matzkin EG, Zeng L. Insulin-like growth factor II (IGF-II) inhibits IL-1beta-induced cartilage matrix loss and promotes cartilage integrity in experimental osteoarthritis. Journal of Cellular Biochemistry. 2015;**116**(12):2858-2869

[42] Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Developmental Cell. 2005;**8**(5):727-738

[43] Reinhold MI, Kapadia RM, Liao Z, Naski MC. The Wnt-inducible transcription factor

Twist1 inhibits chondrogenesis. The Journal of Biological Chemistry. 2006;**281**(3):1381-1388

[44] Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Developmental Cell. 2005;**8**(5):739-750

[45] Guo X, Mak KK, Taketo MM, Yang Y. The Wnt/beta-catenin pathway interacts differentially with PTHrP signaling to control chondrocyte hypertrophy and final maturation. PLoS One. 2009;**4**(6):ew6067

[46] Sato S, Kimura A, Ozdemir J, Asou Y, Miyazaki M, Jinno T, et al. The distinct role of the Runx proteins in chondrocyte differentiation and intervertebral disc degeneration: Findings in murine models and in human disease. Arthritis and Rheumatism. 2008;**58**(9):2764-2775

[47] Stricker S, Fundele R, Vortkamp A, Mundlos S. Role of Runx genes in chondrocyte differentiation. Developmental Biology. 2002;**245**(1):95-108

[48] Wang WJ, Sun C, Liu Z, Sun X, Zhu F, Zhu ZZ, et al. Transcription factor Runx2 in the low bone mineral density of girls with adolescent idiopathic scoliosis. Orthopaedic Surgery. 2014;**6**(1):8-14

[49] Duval E, Bouyoucef M, Leclercq S, Bauge C, Boumediene K. Hypoxia inducible factor 1 alpha down-regulates type i collagen through Sp3 transcription factor in human chondrocytes. IUBMB Life. 2016;**68**(9):756-763

[50] Fernandez MP, Young MF, Sobel ME. Methylation of type II and type I collagen genes in differentiated and dedifferentiated chondrocytes. The Journal of Biological Chemistry. 1985;**260**(4):2374-2378

[51] Haq SH. 5-Aza-2′-deoxycytidine acts as a modulator of chondrocyte hypertrophy and maturation in chick caudal region chondrocytes in culture. Anatomy & Cell Biology. 2016;**49**(2):107-115

[52] Zimmermann P, Boeuf S, Dickhut A, Boehmer S, Olek S, Richter W. Correlation of COL10A1 induction during chondrogenesis of mesenchymal stem cells with demethylation of two CpG sites in the COL10A1 promoter. Arthritis and Rheumatism. 2008;**58**(9):2743-2753

[53] Kumar D, Lassar AB. Fibroblast growth factor maintains chondrogenic potential of limb bud mesenchymal cells by modulating DNMT3A recruitment. Cell Reports. 2014;**8**(5):1419-1431

[54] Shen J, Abu-Amer Y, O'Keefe RJ, McAlinden A. Inflammation and epigenetic regulation in osteoarthritis. Connective Tissue Research. 2017;**58**(1):49-63

[55] Chabane N, Zayed N, Afif H, Mfuna-Endam L, Benderdour M, Boileau C, et al. Histone deacetylase inhibitors suppress interleukin-1beta-induced nitric oxide and prostaglandin E2 production in human chondrocytes. Osteoarthritis and Cartilage. 2008;**16**(10):1267-1274

[56] Young DA, Lakey RL, Pennington CJ, Jones D, Kevorkian L, Edwards DR, et al. Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. Arthritis Research & Therapy. 2005;**7**(3):R503-R512

[57] Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, et al. MicroRNA expression in zebrafish

embryonic development. Science. 2005;**309**(5732):310-311

[58] Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis and Rheumatism. 2009;**60**(9):2723-2730

[59] Nakamura Y, He X, Kato H, Wakitani S, Kobayashi T, Watanabe S, et al. Sox9 is upstream of microRNA-140 in cartilage. Applied Biochemistry and Biotechnology. 2012;**166**(1):64-71

[60] Florine EM, Miller RE, Liebesny PH, Mroszczyk KA, Lee RT, Patwari P, et al. Delivering heparin-binding insulin-like growth factor 1 with self-assembling peptide hydrogels. Tissue Engineering. Part A. 2015;**21**(3-4):637-646

[61] Florine EM, Miller RE, Porter RM, Evans CH, Kurz B, Grodzinsky AJ. Effects of dexamethasone on mesenchymal stromal cell chondrogenesis and aggrecanase activity: Comparison of agarose and self-assembling peptide scaffolds. Cartilage. 2013;**4**(1):63-74

[62] Roach BL, Kelmendi-Doko A, Balutis EC, Marra KG, Ateshian GA, Hung CT. Dexamethasone release from within engineered cartilage as a chondroprotective strategy against interleukin-1alpha. Tissue Engineering. Part A. 2016;**22**(7-8):621-632