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Review

Cartilage tissue engineering: Molecular control of chondrocyte differentiation for proper cartilage matrix reconstruction ☆

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ABSTRACT

Background: Articular cartilage defects are a veritable therapeutic problem because therapeutic options are very scarce. Due to the poor self-regeneration capacity of cartilage, minor cartilage defects often lead to osteoarthritis. Several surgical strategies have been developed to repair damaged cartilage. Autologous chondrocyte implantation (ACI) gives encouraging results, but this cell-based therapy involves a step of chondrocyte expansion in a monolayer, which results in the loss in the differentiated phenotype. Thus, despite improvement in the quality of life for patients, reconstructed cartilage is in fact fibrocartilage. Successful ACI, according to the particular physiology of chondrocytes in vitro, requires active and phenotypically stabilized chondrocytes.
Scope of review: This review describes the unique physiology of cartilage, with the factors involved in its formation, stabilization and degradation. Then, we focus on some of the most recent advances in cell therapy and tissue engineering that open up interesting perspectives for maintaining or obtaining the chondrogenic character of cells in order to treat cartilage lesions.
Major conclusions: Current research involves the use of chondrocytes or progenitor stem cells, associated with "smart" biomaterials and growth factors. Other influential factors, such as cell sources, oxygen pressure and mechanical strain are considered, as are recent developments in gene therapy to control the chondrocyte differentiation/dedifferentiation process.
General significance: This review provides new information on the mechanisms regulating the state of differentiation of chondrocytes and the chondrogenesis of mesenchymal stem cells that will lead to the development of new restorative cell therapy approaches in humans. This article is part of a Special Issue entitled Matrix-mediated cell behavior and properties.

1. Introduction

Articular cartilage is made up of dense, elastic connective tissue localized at the junction of several locations in the skeleton. It covers the surface of the joints to ensure that joints and bones move together. It is an avascular tissue that is not innervated and is composed primarily of a single cell type, the chondrocyte, which synthesizes an abundant extracellular matrix (ECM). Chondrocyte has a hypoxic (physioxic)

Abbreviations: ACI, autologous chondrocyte implantation; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motif; AGEs, advanced glycation end products; AP-2, activator protein-2; BM MSC, bone marrow mesenchymal stem cell; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; Cbfa1/Runx2, core-binding factor subunit alpha-1/Runt-related transcription factor 2; CD, cluster of differentiation; CS, chondroitin sulfate; ECM, extracellular matrix; ESC, embryonic stem cell; GAG, glycosaminoglycan; HA, hyaluronic acid; HAC, human articular chondrocyte; HIF, hypoxia-inducible factor; HtrA1, high temperature requirement A; IGF, insulin-like growth factor; IL, interleukin; iPSC, induced-pluripotent stem cell; KS, keratan sulfate; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; NF-κB, nuclear factor-kappa B; NO, nitric oxide; OA, osteoarthritis; PEG, polyethylene glycol; PG, proteoglycan; PGA, poly(glycolic acid); PLA, poly(lactic acid); PLGA, poly(ε-caprolactone); PTHrP, Parathyroid Hormone-related Protein; ROS, reactive oxygen species; Sox, SRY-type HMG box; TGF-β, transforming growth factor-beta; TNF, tumor necrosis factor; T3, triiodothyronine; T4, thyroxin; UCB MSC, umbilical cord blood mesenchymal stem cell; VEGF, vascular endothelial growth factor

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metabolism and produces mainly type II collagen and aggrecan, the two major phenotypic markers specific to articular cartilage. This collagen isoform imparts resistance to compression forces in cartilage. Aggrecan is a proteoglycan (PG) composed of many sulfated glycosaminoglycan (GAG) chains, such as chondroitin sulfate (CS) and keratan sulfate (KS) and, as a result, it can retain a significant amount of water, providing flexibility and viscoelasticity to the musculoskeletal system. The viscoelastic nature of cartilage tissue confers the ability to absorb pressure and distribute it throughout the articular surface. Additionally, the three-dimensional organization and the nature of ECM molecules are different depending on how deep they are in the cartilage, adding to the complexity of cartilage tissue.

Osteoarthritis (OA), a degenerative disease of articular cartilage, is characterized by the degradation of the ECM, associated with increased secretion of matrix metalloproteinases (MMPs) and aggrecanases [1,2]. Increased intra-articular expression of these ECM-degrading enzymes is triggered by the secretion of pro-inflammatory cytokines in the synovial fluid, such as interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α). These catabolic molecules disrupt the integrity of the ECM and decrease the response and sensitivity of chondrocytes to external anabolic signals [3]. In addition, the OA process induces chondrocyte dedifferentiation characterized at least in part by increased synthesis of type I collagen, an atypical isoform in articular cartilage [4]. Moreover, due to the poor intrinsic healing capacity of articular cartilage, there is currently no treatment to restore the chondrocyte phenotype and, in the most advanced stages of OA, the joint must be replaced with a prosthesis, requiring surgery.

Therefore, various drug and surgical treatments have been developed in an attempt to prevent the destruction of cartilage which, in light of their relative success, then lead to new, improved therapeutic strategies. One of the most promising approaches is based on articular cartilage tissue engineering based on the procedure described by Brittberg et al. using autologous chondrocyte implantation (ACI) [5]. Applied in the earliest stages of OA or chondral lesions, ACI is based on the use of chondrocytes from a healthy, non-bearing region of the diseased joint. The cells are then amplified in monolayer culture and then re-implanted in the lesion. However, amplification of autologous chondrocytes in two-dimensional culture mimics, at least in part, some of the characteristics of the OA process and is accompanied by cell dedifferentiation leading to the formation of non-functional fibrocartilage.

The numerous pharmaceutical approaches and surgical techniques developed to repair cartilage lesions have revealed their limitations. Ideally, traumatic cartilage lesions should be treated earlier to prevent OA and postpone prosthetic surgery. In the interest of preventing OA, cartilage cell therapy has proven to be a pivotal approach for repairing damaged tissue. Cell therapy consists not only in filling the cartilage lesion with healthy chondrocytes, but also in reconstituting the structure, the physico-chemical properties and the functionality of the hyaline matrix. The transplantation of autologous chondrocytes is the foundation of cell therapy and there have been several generations of ACI, each improving on the previous one. However, even the most recent ACI techniques are beginning to show limitations.

Consequently, research efforts are now focused on improving this technique to obtain, after amplification, a differentiated and stable chondrocyte phenotype using new types of biomaterials, molecules known for their chondrogenic activity, RNA interference strategies and different cell sources. Human articular chondrocytes (HACs) and/or human mesenchymal stem cells (MSCs) are employed to restore or maintain the chondrocyte phenotype and induce chondrogenesis, respectively. Today, adult MSCs hold much promise for biomedical research because they are able to reform many tissues, including cartilage. However, the culture conditions used to obtain a differentiated chondrocyte phenotype must be well defined. The nature of the medium, the amount of oxygen or the presence of pro-chondrogenic factors, the use of different biomaterials, and the gene therapy strategy are all parameters that need to be considered to stabilize the differentiated chondrocyte phenotype.

As a result, we present some of the most recent advances in the field of cartilage engineering and the critical parameters for which more research is required to better understand the mechanisms regulating the state of differentiation of cartilage cells and chondrogenesis of adult MSCs. Knowledge in these areas will most probably lead to new restorative cell therapies for human medicine.

2. Articular cartilage

2.1. Adult articular cartilage homeostasis

Adult articular cartilage is composed of chondrocytes which are encapsulated in a dense pericellular matrix. It contains a high concentration of PGs and type VI collagen for anchoring cells to the ECM [6]. Chondrocytes do not divide and apoptotic activity is very low [7,8]. In adult articular cartilage, chondrocytes are not organized in columns, but appear to be uniformly isolated in the tissue. New chondrocytes are probably produced by successive mitosis and not via the initial chondrogenesis process. In addition, the metabolic activity of chondrocytes from adult cartilage is decreased. Articular cartilage had long been considered as a tissue containing only one cell type, the chondrocyte. However, we now know that MSCs and/or progenitor cells are also present in this tissue and have the capacity to differentiate into mature articular chondrocytes [9]. These cells may thus also contribute to the local repair of micro-lesions in articular cartilage [10].

Within the cartilage, chondrocytes are subjected to numerous mechanical and environmental factors that regulate their metabolic activity and phenotype. Thus, according to the different signals they perceive, chondrocytes are responsible for the production, organization and maintenance of the integrity of cartilage ECM.

2.1.1. The extracellular matrix (ECM) of cartilage

The nature of the ECM in adult articular cartilage depends on the location of chondrocytes. Chondrocytes are surrounded by a pericellular matrix covered in turn by a territorial matrix. These two matrix layers bind chondrocytes to the interterritorial matrix and serve to protect against potential cartilage injuries. The interterritorial matrix fills the cell-free matrix space and occupies the greatest volume in cartilage. The chondrocyte is anchored to the ECM via cartilage PGs and membrane proteins such as syndecan [11] or glypicans [12]. Transmembrane proteins are also involved in anchoring the chondrocyte to its pericellular space, including anchorin CII, which interacts with cells possessing type II collagen, and integrins, which attach chondrocytes to collagen molecules and fibronectin [13].

Cartilage ECM is mainly composed of collagen (60% of dry weight), PGs (5–10%), including in particular a specific marker, aggrecan, and non-collagenous proteins. Among the different types of collagen, the type II isoform, a homotrimer composed of an α1 (II) chain, is the most abundant isoform in articular cartilage, representing 80% of collagen. The remaining isoforms include type IX and XI collagens (15%) and 5% of other collagen types (types III, XII, VI, etc.) [14]. Given its abundance in the ECM space, type II collagen is the leading marker specific to chondrocytes along with aggrecan. Its role in ECM homeostasis is critical. Its loss induces perturbation of the physico-mechanical properties of cartilage and leads to OA. Moreover, type II collagen −/− mice have a disorganized ECM, without any collagen fibers. The mice are smaller and have a much less developed skeleton [15]. Large amounts of water molecules (65–80%) are found in the ECM and the dry weight of the ECM represents 20–40% of cartilage volume. Chondrocytes are responsible for maintaining cartilage homeostasis but the nature of the ECM can also affect their behavior and phenotype. Alteration of the ECM or degradation of its components inevitably leads to chondrocyte dedifferentiation. The arrangement of molecules in this three-dimensional network thus maintains chondrocytes in a differentiated

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