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Investigating the Effectiveness of Stem Cells in Cartilage Tissue Engineering

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Abstract

Cartilage is a tissue without blood vessels and lymph, and extensive damage causes it to lose its ability to repair and regenerate. Cartilage diseases, including cartilage damage and arthritis, are increasing day by day. The damage to the cartilage causes the daily functioning of the patients to be disturbed and causes pain in the patient through wear of the bones. Common methods used to treat invasive cartilage injuries have low efficacy and include implantation of one's chondrocytes, microfracture, bone marrow stimulation, and removal of the damaged part. Common treatments are usually not definitive methods and stem cells and cartilage tissue engineering are used. The purpose of this study was to review stem cells used in cartilage cell therapy and cartilage tissue engineering. Also, in this study, cell messenger factors such as growth factors, and mechanical and environmental factors were investigated. According to the results of this study, stem cells are effective in repairing cartilage, but the mechanism and method of creating this repair have not been precisely determined. The highest security in the use of cell therapy in cartilage was related to mesenchyme cells and the most clinical use of these types of cells. Cell therapy for patients is done clinically, but cartilage tissue engineering has a long way to reach the clinical stage.

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Introduction

Articular cartilage is one of the special tissues of the body because it does not have any vessels, nerves, or lymph. There is a wide range of treatment methods for cartilage damage, and the required method depends on the location, extent, shape, depth of the damage, and the age of the patient (Simon & Jackson, 2006; Liu *et al*., 2021; Roseti & Grigolo, 2022). Common and old methods for the treatment of cartilage injuries include micro-fissure, fragment removal, drilling, abrasion, chondrocyte, and osteochondral grafting (iron grafting and plum grafting). These methods are associated with relative recovery and reduction of symptoms in patients, but until now the tissue created by these methods has been fibroblastic cartilage tissue, which is mechanically weaker than normal cartilage tissue. A more up-to-date strategy is to use the individual's chondrocytes as implants (ACI) and biological or synthetic matrices that can be combined with cells (stem cells and chondrocytes) and chemical factors such as growth factors or physical-mechanical factors (Makris *et al*., 2015; Francis *et al*., 2018).

Cartilage is naturally composed of interconnected mesenchymal cells and the extracellular matrix secreted from them. These cells become organized over time and when the matrix is separated from the cells it is known as cartilage. Over time, the cells change into a cylindrical shape. As cartilage tissue matures, cells lose their multiline age ability, and new chondrocyte cells cannot migrate, multiply, and participate in repair. In addition, mature chondrocytes have little ability to secrete extracellular matrix. These characteristics of the specific cells of the cartilage cause the spontaneous repair and regeneration of this face do not take (Simon & Jackson, 2006; Liang *et al*., 2022; Lee *et al*., 2022).

Common treatment methods along with surgery include cultivating and implanting the patient's chondrocyte cells, for this purpose, cells are taken from the individual, and chondrocyte cells are separated by enzymes and cultured in the environment outside the body until the number reaches the target Then they are placed on a synthetic scaffold and implanted in the body, or a person's healthy cartilage is used as a scaffold. Another common treatment method is to stimulate the bone marrow with methods such as drilling or creating a gap to eventually induce stem cells in the damaged area (Simon & Jackson, 2006; Guan & Hou, 2022). Most of these methods do not have a long-term response, and the patient suffers secondary cartilage damage after a while, they turn to tissue engineering and regenerative medicine to solve these problems, but

the absence of a supporting scaffold at the site of the injury makes the efficiency of the method less effective. Also, because the scaffold is not present on the site, the differentiation of the stem cells of the tissue itself is facing a problem, and today, the treatment method is moving towards the use of scaffolds to solve this problem.

The advantages of using a scaffold are better filling of the injured area, less damage to the healthy tissue of the person, less challenge in the grafting with healthy tissue, and less recovery time. Conventional cell-based clinical methods for the treatment of cartilage injuries and cartilage regeneration are based only on chondrocyte cells. It has been done mechanically and biochemically. During the experiments, to reduce the disadvantages of chondrocyte cultivation, the tendency has gone towards the use of stem cells, and many of these methods are in line for approval from the American Food and Drug Administration (Makris *et al*., 2015; Ma *et al*., 2017; Shestovskaya *et al*., 2021; Guo *et al*., 2023).

In this review article, the stem cells used in the treatment of cartilage injuries, their better differentiation methods to chondrocytes, clinical studies conducted in this field, and cartilage tissue engineering were introduced. Therefore, the current research aims to review the use of stem cells in cartilage tissue repair and engineering.

Clinical Use of Stem Cells in the Treatment of Cartilage Diseases

Compared to embryonic and induced stem cells, adult stem cells are safer for treatment, and for this reason, the first clinical application is specific to these cells (Wang *et al*., 2017). Clinical results for the use of mesenchymal stem cells for the treatment of cartilage damage in humans have not been mentioned in many articles, however, there has been an upward growth. Because the cell source, treatment formula, cell delivery method, and clinical indicators are completely different from one study to another. It is difficult to compare the efficiency of each study and reach a general conclusion.

According to recent clinical methods based on cell therapy, the most used cells are bone marrow, synovial, and fat tissue cells, which can be placed in the body through one-step injection or two-step implant. Tests to follow the treatment process include histology tests, magnetic resonance imaging, and arthroscopic evaluation (Van Pham, 2016). In general, the use of mesenchymal stem cells compared to conventional methods of treating cartilage damage, such as implanting one's chondrocyte cells, has many advantages, including the fact that there is no need to biopsy healthy cartilage, and as a result, secondary damage to the patient. One of the cell therapy methods that is performed clinically is combining mesenchymal cells with platelet-rich plasma, which is taken from the patient and reduces the possibility of stimulating the immune system. These cells are specific to the patient's body, but they need to undergo an operation before being injected into the site. This process involves separating mesenchymal cells by centrifugation and purifying them to increase the differentiation to chondrogenesis and the expression of type 2 collagen and glycosaminoglycan (Uth & Trifonov, 2014). For clinical application, in a study, the bone marrow cells of an athlete were used to regenerate his femoral cartilage damage. The cells were placed in the collagen scaffold and implanted together with the periosteal pieces at the injury site. 7 months after surgery, the results obtained from arthroscopy and histology have confirmed the creation of articular cartilage tissue (Van Pham, 2016). Search results of pre-clinical research were in the Pubmed database and clinical results were presented in the clinicaltrial.gov database (Anderson *et al*., 2014).

Cartilage Tissue Engineering by Stem Cells

Tissue engineering is a broad field with high potential for permanent treatment of cartilage injuries. Cartilage tissue engineering strategies consist of three main elements: cell source, biomaterials for scaffolds, and biochemical/biophysical stimuli to induce growth and tissue formation (Vinatier & Guicheux, 2016).

Cartilage Tissue Engineering Scaffold

Scaffolds used in cartilage tissue engineering can be divided into synthetic and natural. Natural scaffolds such as collagen, fibrin, alginate, chitosan, silk, and cellulose (Mirahmadi *et al*., 2013) or hyaluronic acid have optimal biocompatibility and biodegradability and can maintain the phenotype of cells close to their natural state, while polymer Synthetics such as PGA, PEG, and PLAY have minimal species diversity, but they need modifications to improve their biocompatibility and biodegradability properties. An ideal scaffold, in addition to its biocompatibility and biodegradability properties, should be able to provide a suitable three-dimensional environment for the cells so that the cells can maintain their chondrocyte phenotype. In addition, it must be permeable to the molecules of nutrients and growth factors. Hydrogels, which are composed of a polymer network, can absorb water at a high rate and even more than their weight and are considered a suitable option for cartilage tissue engineering scaffolds. Because they can deliver nutrients to the place without cartilage vessels. Various matrices used in cartilage tissue engineering were prepared (Vinatier & Guicheux, 2016).

Among natural scaffolds, scaffolds based on hyaluronic acid are considered the most trusted scaffolds for cartilage tissue engineering in recent decades. It is worth noting that polymer scaffolds can be mesh or sponge for implanting and hydrogel for direct injection with less invasiveness. Scaffolding biomaterials are also used as carriers for the release of active molecules such as growth factors. These bioactive molecules can be physically trapped inside the scaffold or covalently linked to the scaffold (Solouk *et al*., 2014; Richardson *et al*., 2016; Vinatier & Guicheux, 2016). The chemical composition of the scaffolds used in cartilage tissue engineering is also very important and can affect the differentiation of stem cells.

Cartilage Tissue Engineering Cells

Until now, chondrocytes, fibroblasts, and stem cells have been used for cartilage tissue engineering, and chondrocytes and mesenchymal stem cells have received the highest studies. Various scaffolds and carriers have been used to use chondrocyte cells to treat cartilage injuries, but hydrogels are one of the most reliable options as scaffolds for cartilage tissue engineering. In vitro,

studies have proven that chondrocytes proliferate and spread well in three-dimensional hydrogel matrices, and as a result, the gene related to the production of cartilage proteins is expressed, and they maintain the original phenotype and morphology of a healthy chondrocyte. Hydrogels depending on their constituent polymers activate different chondrogenesis processes. For example, chitosan and agarose hydrogel both have long-term cell viability and good morphological preservation for chondrocyte cells, and during cell culture and maturation of cells in vitro, chondrogenesis and creation of cartilage extracellular matrix containing collagen type 2 and Aggrecan occurred in the mentioned hydrogels (Jin *et al*., 2009).

Although the use of chondrocytes is a suitable option in the treatment of cartilage defects, there are also notable limitations. The first limitation is that the chondrocyte must be extracted from healthy cartilage tissue without applying load, and then it must be cultured in vitro for a long period of two to three weeks. Due to the small number of chondrocyte cells in the cartilage, the damaged cartilage cannot repair itself, and the place from which the cell is extracted is considered an unhealthy tissue. The second limitation is that the extraction of chondrocytes from the individual has a low yield for the elderly because the extracted chondrocytes do not have a high proliferative capacity (Giannoni *et al*., 2005).

Co-cultivation of chondrocytes and mesenchymal stem cells in hydrogels is also done to overcome the limitations of using cells alone. Ko *et al*. (2016) cultivated chondrocytes and mesenchymal stem cells with different population ratios in PCL-PEG composite hydrogel in the presence of culture medium and implanted them in rabbits with articular cartilage defects. The in vitro results during 4 weeks have indicated that the co-culture of chondrocyte and mesenchymal stem cells facilitates the expression of cartilage phenotype and the production of extracellular matrix. Also, the presence of chondrocytes encourages differentiation of stem cells into chondrocytes, while stem cells increase cell proliferation. In vivo, results during 8 weeks indicated that the co-culture of a chondrocyte, mesenchymal stem cell with a rate of 1:4, had the most optimal cartilage production.

Sheehy *et al*. (2013) studied the bilayer co-culture of chondrocytes and MSCs in agarose hydrogels for osteochondral tissue repair. In this design, chondrocytes are placed in the first layer of agarose hydrogel (as the cartilage layer), while in the lower layer, mesenchymal stem cells are placed (as the bone layer). This bilayer construct induces chondrogenesis in the cartilage layer, facilitates the expression of the cartilage phenotype, and also delays hypertrophy and mineralization in the bone layer. The results of the subcutaneous implant of this hydrogel show the formation of osteogenesis in the bone layer and the production of osteochondral tissue. Osteochondral layered hydrogels containing all types of chondrocyte cells and stem cells can induce osteogenesis, chondrogenesis, and regional osteochondral tissue production. Therefore, such hydrogels are suitable choices for the treatment of cartilage and osteochondral tissue damage. If these systems are combined with biochemical stimuli, they will have more reproductive effects (Yang *et al*., 2017).

Messenger Factors in Cartilage Tissue Engineering

As mentioned, stimulating factors can be chemical, physical, mechanical, and geometric factors (Vinatier & Guicheux, 2016). The natural extracellular matrix of cartilage causes the proliferation, differentiation, and growth of cells through growth factors. These growth factors and hormones used in cartilage tissue engineering are transforming growth factor (TGF), bone morphogenetic protein (BMP), insulin-like growth factor (IGF), and dexamethasone (Yang *et al*., 2017).

TGFs are a family of polypeptides that can affect cell behaviors including growth, proliferation, and differentiation. There are two types of TGFs, α (alpha) and β (beta), each of which has a unique amino acid sequence and a different interaction with the receptor. The β family can differentiate stem cells into cartilage, and β1 and β3 are widely used for the differentiation of MSCs into chondrocytes and cartilage tissue regeneration, although their effects are not the same. β1 has been reported to have higher cartilage-related gene expression than β3. β1 promotes cell adhesion and cell density, while β3 promotes cell proliferation. It is worth noting that β1 and β3 each participate in different stages of MSC differentiation to cartilage, so a combination of both is needed for greater effect (Lu *et al*., 2014a).

IGFs are single-stranded polypeptides that have the same sequence and amino acid sequence as insulin. There are two types of IGF including IGF-1 and IGF-2. IGF-1, which is secreted by the liver controls growth in adults. IGF-2 is believed to play an essential role in the growth of the fetus. IGF-1 is widely used for cartilage treatment because it plays an essential role in cartilage homeostasis and moderating the synthesis of proteoglycans. In research, the expression of IGF-1 was increased by grafting alginate hydrogel to repair cartilage defects in rabbits. The results have shown that within 14 weeks, it improves the reproduction of articular cartilage and accelerates the formation of subchondral bone. In addition, the presence of IGF-1 in the hydrogel improves the chondrogenic properties in vitro and in vivo. IGF can regenerate cartilage with high efficiency by chondrocytes (Madry *et al*., 2005; Lu *et al*., 2014b).

In the case of BMPs, Euryst first discovered that the active components responsible for bone regeneration are a family of proteins called BMPs (Urist, 1965). These types of proteins can stimulate the production of cartilage and bone so that bone production occurs similarly to the embryonic state. So far, nearly 20 members of this family have been identified, and 1, 5, 9, 13, and 14 play an essential role in cartilage production and the differentiation of mesenchymal stem cells into cartilage (Xiao *et al*., 2007). Some studies have shown that BMPs-2 can produce cartilage from mesenchymal stem cells and chondrocyte cells, and this is very important for the treatment of cartilage and osteochondral injuries (Park *et al*., 2005).

Dexamethasone is an adrenal hormone that induces stem cell differentiation into cartilage. Dexamethasone is added to the culture medium or covalently attached to the hydrogel to induce stem cell differentiation (Tanaka *et al*., 2004). It has been reported that dexamethasone, in addition to facilitating the differentiation of mesenchymal stem cells and embryonic stem cells into cartilage cells, also can produce cartilage-related proteins such as TGF-B

(Florine *et al*., 2013). However, another study reported that increasing the concentration of dexamethasone suppresses the expression of aggrecans (Awad *et al*., 2003). Dexamethasone is usually used together with TGF or BMP to maximize cartilage repair (Yang *et al*., 2017).

Striking and continuous efforts have been made to identify new growth factors that affect cartilage and bone differentiation. For example, a small molecule called cartogenin has been reported to induce chondrocyte differentiation by regulating the transcription of genes. In addition, some inorganic particles (micro and nano) have a function similar to growth factors in cell differentiation (Johnson *et al*., 2012; Xavier *et al*., 2015).

Conclusion

The role of stem cells in cartilage repair has been proven correctly, but the mechanism and method of this repair have not been determined yet. Mesenchymal cells have the highest security in the use of cell therapy in cartilage, and these types of cells have the most clinical use. Cell therapy for patients is done clinically, but cartilage tissue engineering has a long way to reach the clinical stage.

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