

RECENT ADVANCES IN TISSUE ENGINEERING APPROACHES FOR ARTICULAR CARTILAGE REGENERATION

Abstract

Cartilage is a specialized connective tissue present in different parts of the human body as well as also found in animals, too. Some of the major functions of cartilage include the ability to withstand compressive forces, improve bone resistance, and provide flexibility and support for easy gliding of joints and bones. Despite these important functions, it is one of those critical tissues that is devoid of blood vessels, nerve endings, and the lymphatic system. This results in poor healing of the cartilage, if damaged. The total incidence rate of cartilage defects is higher in older people and young athletes. The current treatment modalities focus on the use of medications and a few restorative therapies that are invasive and do not restore the cartilage structure completely leading to pain and osteoarthritis if left untreated. Tissue engineering strategies look promising due to the use of living cells, growth factors, and/or bioactive agents or scaffolds to replace damaged or diseased tissue. This book chapter emphasizes the current and latest developments in tissue engineering techniques involving surgical interventions, the use of stem cells, growth factors, bioactive agents, and 3D bioprinting used for cartilage restoration, regeneration, and repair.

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I. INTRODUCTION

Articular cartilage (AC) is a specialized connective tissue that lines the bony surfaces allowing them to glide with each other. It is present in different parts of the body such as the ear, nose, rib cage, bronchial tubes, intervertebral discs, meniscus, and the joints between bones¹. Some of the important functions of cartilage include the ability to withstand compressive forces, improve bone resistance, and provide flexibility and support for easy gliding of joints and bones. The major cell type that makes up the cartilage is known as the 'chondrocyte', that is present in the bone lacunae. The extracellular matrix (ECM) of AC is made up of a collagenous network that primarily consists of type II collagen, various combinations of glycosaminoglycans (GAG) (for example, hyaluronan), and a variety of proteoglycans (like GAG-containing proteins)^{2,3}. Despite being structurally tough, AC has a limited healing capacity due to restricted blood supply, lack of nerve endings, and a lymphatic system. Owing to this, AC defects continue to be a critical clinical problem for physicians and orthopedic surgeons. The total incidence rate of these defects is found to be higher in older people and young athletes⁴.

The current mode of treatment includes medications (acetaminophen and ibuprofen for symptomatic relief), restorative therapy such as microfracture, mosaicplasty and osteochondral allografts or autografts (OAT), and regenerative therapy like autologous chondrocyte implantation (ACI)⁵. Most of the restorative and regenerative treatments have shown beneficial results, however, they do not restore the AC defects entirely preceding pain and early osteoarthritis (OA). Appropriate graft availability and donor site morbidity also restrict the use of such techniques, convincing the researchers to look for other novel options that can treat AC defects non-invasively.

Tissue engineering (TE) approaches have gained widespread interest due to their potential to restore/replace damaged tissues thereby affecting disease progression⁶. This field offers numerous possibilities for transforming various debilitating diseases, including AC defects. It is proposed to regenerate a tissue that is structurally and mechanically like the native cartilage, unlike fibrocartilage⁵. TE utilizes living cells, growth factors, and/or bioactive agents or scaffolds to replace damaged or diseased tissue. Successful tissue regeneration relies on many factors for instance the type of cell source being employed, the use of appropriate growth factors/biological active agents to direct differentiation to a particular lineage, and a three-dimensional (3D) matrix that supports the dividing cells until the tissue is completely restored¹. This chapter emphasizes the current and latest developments in TE techniques involving surgical interventions, the use of stem cells, growth factors, bioactive agents, and 3D bioprinting used for AC restoration, regeneration, and repair.

II. SURGICAL ADVANCES FOR AC REGENERATION

Cartilage defects usually are accompanied by severe pain, stiffness, and immobility and, if left untreated can result in osteoarthritis in the long term⁷. The major objective of cartilage restoration is to stimulate new cartilage growth or implant new cartilage in the damaged area. Surgical techniques are often recommended when other conventional treatments like medicine or physiotherapy don't help. Several new surgical techniques have

been developed in the past decade, most of which are minimally invasive and utilize an arthroscope (an endoscope used for joints)⁸.

- 1. Chondroplasty (Cartilage Debridement):** Chondroplasty, also known as “cartilage debridement,” is a first choice for treating minor cartilage issues. This technique involves removing loose flaps of cartilage (which causes joint pain) or fragments of tissue, as well as smoothing damaged areas⁹. With the help of small surgical tools (usually an arthroscope), a surgeon removes the damaged cartilage and any loose tissue around the affected area. The excess fluid is removed from the knee joint, and the incisions are sealed, thereby allowing a new ‘scar’ tissue to replace the cartilage defects. Recovery from this technique is much faster than traditional open surgery.
- 2. Microfracture:** Microfracture is used for treating symptomatic AC defects in the knee joint by creating a new blood supply. This technique was introduced by Steadman and his colleagues in the early 1980s¹⁰ and since then, it has become the first line of treatment for surgeons. The surface of the bone, known as subchondral bone, is hard and has limited blood flow, hence tiny fractures are created with arthroscopic surgical awls till it reaches the bone marrow. The holes are in general between 2 to 4 mm apart, depending on the lesion size which eventually forms a clot known as a super clot (Figure 1). It is believed that bone marrow stem cells aid in the regeneration of cartilage from the super clot¹¹. This process is only effective for short-term relief as it regenerates another type of cartilage called fibrocartilage which may degenerate over a period and other advanced techniques are required to address the above issue.
- 3. Osteochondral Autologous Transplantation System (OATS)/Mosaicplasty:** Osteochondral Autologous Transplantation System (OATS) also known as “mosaicplasty”, is commonly utilized for treating small, 2- to 3-cm lesions. In this technique, small cylindrical plugs are removed from a non-weight-bearing region of the patient itself (whose functions are non-vital to the body) and implanted in a focal chondral defect to restore cartilage function and provide pain relief to a load-bearing region of the knee joint¹² (Figure 1).
- 4. Osteochondral Allograft Transplantation (OATS):** If the cartilage defect is too large for an autograft to restore, an allograft may be considered. Unlike autografts, allografts are taken from a cadaveric donor. The allograft tissue is sterilized, prepared in the laboratory, and tested for any possible diseases that might be transmitted to the recipient before being inserted into a focal chondral defect¹³ (Figure 1).
- 5. Matrix-Assisted Chondrocyte Implantation (MACI):** Matrix Assisted Chondrocyte Implantation (MACI) is useful for patients who have defects above 2 cm in diameter. It is a two-step procedure in which new autologous cartilage cells (chondrocytes) are proliferated and then placed in the cartilage defect. Using an arthroscope, a small part of cartilage is removed from a non-weight-bearing area of the bone and the healthy chondrocytes are propagated on a collagen matrix in a cell culture laboratory until the number increases over 1 month. An open surgical procedure is then performed to implant the newly cultured chondrocytes within the defect and the defect is sealed using fibrin glue as shown in figure 1 (a biological adhesive)¹⁴.

6. Autologous Matrix-Induced Chondrogenesis (AMIC): Traditional surgical techniques have certain limitations. One of that is the inherent stem cells (SCs) and growth factors ooze out of the joint rather than being localized. AMIC is a novel one-stage technique that has been recently developed to address the above issue. It is a combination of microfracture and the addition of collagen matrix to help repair and regain full mobility of the joint. Tiny fractures developed using microfracture are covered using a collagen matrix that allows mesenchymal stem cells (MSCs) and growth factors to proliferate in a three-dimensional (3D) structure and reside within the joint and regenerate¹⁵.

III. STEM CELLS FOR CARTILAGE REGENERATION

Stem cells (SCs) hold remarkable properties to differentiate into various kinds of cells there by serving as a repair mechanism for many tissues of the body¹⁶. SCs possess two unique features which distinguish them from other types of cells. First, stem cells possess self-renewal properties and can differentiate multiple times through cell division,¹⁷ and second, they can be redirected to differentiate into a particular lineage¹⁸. In this chapter, we will discuss mesenchymal stem cells (MSCs) which are largely explored for cartilage regeneration and other TE purposes, too. Other SC sources like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) possess great potential clinically, however, have limited exposure due to ethical constraints.

1. Mesenchymal stem cells (MSCs): Mesenchymal stem cells (MSCs), also described as stromal cells, are non-hematopoietic, self-renewing multipotent cells. They were isolated from mouse bone marrow by Friedenstein *et al* for the first time in 1966 who initially described them as colony-forming unit fibroblast (CFU-f)¹⁹. Later, MSCs were harvested from human bone marrow by Pittenger *et al* and proved their potential to differentiate into various tissues like bone, cartilage, fat, tendon, and muscle²⁰. Subsequently, several studies reported MSC isolation from various tissues of an adult body, and soon MSCs became a superior choice for cell-based therapies due to their tri-lineage differentiation ability, immunomodulatory property, and bio-homing capability²¹.

- **Bone Marrow Mesenchymal Stem Cells (BMSCs):** Bone marrow-derived mesenchymal stem cells (BMSCs) have been significantly explored for various therapeutic purposes owing to rapid division, and sustainable differentiation capability. The first clinical study for AC treatment was carried out long ago by Wakitani *etal*. This study involved 24 patients out of which half of them received autologous MSCs injections whereas the others served as case controls. After 42 weeks of transplantation, the treatment group showed regeneration of native hyaline-like cartilage proving the efficacy of BMSCs²². In a similar study with OA patients received a single dose of BMSCs in the knee joint. After 24 weeks, the knee joint showed improvement concerning joint pain and mobility without any supplemental drugs. There was significant cartilage growth which was confirmed by MRI and other clinical parameters²³. Another 5-year-long study of autologous BMSCs transplantation in four patients with moderate to severe OA exhibited improvement in walking, climbing stairs, and joint pain²⁴. Most of the studies so far have utilized autologous MSCs from patients/non-healthy donors, which directly affects MSC functionality and

impacts their proliferation rate and tri-lineage differentiation²⁵. Thus, researchers are now trying to utilize allogenic MSCs due to the higher potential of recovery with biologically fit cells that maximizes the result making it cost-effective. A study performed in 2014 with 55 patients received an intra-articular injection of 2 different doses of allogenic BMSCs and followed up for 2 years. The patients showed improvement in joint pain and movement and no adverse effects were observed until follow-up²⁶. Allogenic BMSCs were also received by the patients recruited for a Phase I clinical trial along with 10% of autologous BMSCs²⁷. After 12 months, the patients did not show any adverse effects and proved that allogenic BMSCs are safe to use thereby opening new avenues to investigate.

- **Adipose-Derived Mesenchymal Stem Cells (AD-MSCs):** AD-MSCs are the most studied cell source after BMSCs due to their higher proliferation and chondrogenic differentiation capabilities than the former²⁸. AD-MSCs are routinely cultured from the lipo-aspirate that is generally discarded after every liposuction surgery. The early method of isolation was developed by Rodbell *et al* back in 1966 from rat fatty tissues and the protocol is employed for the isolation of AD-MSCs from human adipose tissues, as well²⁹.

AD-MSCs can be harvested in bulk quantities with a less invasive procedure when compared to BMSCs. A recent study showed improvement in cartilage regeneration in OA-induced rats in 14 days after administrating with a stromal vascular fraction (SVF) and AD-MSCs³⁰. Another study which involved 60 white rabbit knees were injected with intra-articular or adherent AD-MSCs and the results were obtained after 4-, 12-, and 24 weeks post-transplantation. It was concluded that adherent AD-MSCs were more effective in promoting cartilage regeneration than intra-articular injection³¹. Numerous clinical trials have also confirmed the efficacious effects of AD-MSCs for therapeutic purposes. A clinical trial with 80 patients in the age group of 18-50 with moderate to severe OA received an injection of AD-MSCs and showed improved pain and symptoms scores and improved quality of life³² after 24 months of follow-up. Another phase-IIb clinical trial was carried out in 24 patients which demonstrated improved function and reduced pain after the treatment with AD-MSCs post-six months suggesting the therapeutic ability of AD-MSCs for TERM purposes³³.

- **Peripheral Blood-Derived Mesenchymal Stem Cells (PB-MSCs):** The first-ever use of PB-MSCs in OA was shown by Saw *et al* in 2011 in 5 patients who underwent microfracture surgery and were injected additionally with PB-MSCs post-operatively. The patients did not show adverse reactions and cartilage regeneration was observed³⁴. A similar study in early OA patients showed short-term clinical improvement using PB-MSCs³⁵. Another clinical trial with 52 patients injected with PB-MSCs concluded that large cartilage lesions can be effectively treated with blood stem cells and can be a substitute for ACI³⁶. A lot of research is still required to prove the efficacy of PB-MSCs for TE projects.
- **Synovium-Derived Mesenchymal Stem Cells (SD-MSCs):** SD-MSCs have been isolated from the synovium of the knee, and human synovial fluid using a protocol published in 2001 by De Bari *et al*³⁷. It has become a promising cell source for cartilage regeneration because of its

higher chondrogenic potential than any other MSC source³⁸. An animal study using autologous SD-MSCs was administered in micro mini pigs and after 12 weeks post-transplantation, cartilage regeneration was observed in the knee joint³⁹. Xenogenic transplantation of human SD-MSCs into the canine OA model depicted cartilage restoration, ECM synthesis, and anti-inflammatory response⁴⁰. Another implantation of xenogenic equine SD-MSCs into rat AC defect showed successful regeneration⁴¹.

IV. GROWTH FACTORS (GFs)

Growth factors (GFs) play a vital role in cartilage regeneration and thus are an essential factor of TE research. A major part of GFs is to stimulate chondrogenesis in stem cells, regulate chondrocyte cell division, and subsequently repair AC damage. This chapter includes the most widely used GFs that are used for cartilage regeneration.

- 1. Transforming Growth Factor (TGF- β):** TGF- β is a multi-functional cytokine that is majorly found in platelets and bone. It is the most potent GF known to date and is known to regulate cellular growth, and differentiation along with the synthesis of extracellular matrix (ECM) and apoptosis. In humans, five major types of TGF- β (TGF- β 1- β 5) have been discovered that effectively signal stem cells to differentiate into a chondrogenic lineage⁴². Amongst all, TGF- β 1 is a widely studied GF and is responsible for inducing stem cells to chondrogenic differentiation as well as stimulating the development of type-II collagen and proteoglycan which maintain the chondrocyte phenotype. Along with this, TGF- β 1 is also known to counteract the inhibitory (inflammatory) effect of IL-1 making it a superior GF for the treatment of OA⁴³. TGF- β 3, on the other hand, is known to promote the synthesis of ECM and has been studied in vitro for rabbit cartilage injury⁴⁴.
- 2. Bone Morphogenic Proteins (BMPs):** BMPs are a part of the TGF- β superfamily and have an important task in the development of cartilage and bone. These proteins are involved in multiple signaling pathways and assist in the differentiation of MSCs towards osteogenic lineage. There are currently 20 BMP molecules known so far out of which BMP-2 and BMP-7 are well studied. It is stated that BMP-2 is over-expressed in OA and is hardly seen in healthy cartilage. A study showed that BMP-2 stimulated the synthesis of a new ECM in a cartilage model induced by interleukin 1 beta (IL-1 β)⁴⁵. BMP-7 [also referred to as osteogenic protein 1 (OP-1)] is also known to stimulate ECM synthesis in both SD-MSCs and BMSCs, respectively^{46,47}.
- 3. Cartilage-Derived Morphogenetic Proteins (CDMPs):** CDMPs belong to the BMP family, which are essential during embryonic skeletal development⁴⁸. The CDMP family contains CDMP-1, 2, and 3 which are involved in the synthesis of proteoglycans from chondrocytes. A study reported both the osteogenesis and chondrogenic differentiation of fibroblasts proving its role in cartilage regeneration⁴⁹. An additional study injected the *cdmp-1* gene along with BMSCs in rabbits' knees and 8 weeks post-transplantation, hyaline cartilage, and the surface bone was regenerated⁵⁰.
- 4. Fibroblast Growth Factors (FGFs):** FGFs are a group of cell-signaling molecules produced by macrophages; they are involved in critical processes during animal cell development. Apart from this, FGFs regulate the division and proliferation of fibroblasts,

promote healing of bone and cartilage and induce cell differentiation. FGF-2 (also called basic FGF) and FGF-18 are considered important molecules for cartilage regeneration and OA. An *in vivo* study showed successful regeneration of cartilage, suggesting its therapeutic applications⁵¹. Another study in rat models concluded that FGF-18 induces chondrogenesis in a dose-dependent fashion⁵². In a recent study, human recombinant FGF-18 was used for the treatment of articular cartilage⁵³.

- 5. Insulin Growth Factors (IGFs):** IGFs are single-chain polypeptides with homology to insulin structure. These proteins are regulated by growth hormones and were the first ones to be recognized for chondrogenic potential. IGFs are important for maintaining chondrocyte homeostasis in blood and in synovial fluid and for regulating proteoglycan and its decomposition, *in vitro*. IGF-1 and IGF-2 are two proteins in the IGF family. A study depicted that overexpression of IGF-1 can dramatically improve cartilage damage around the knee area in OA⁵⁴. Another similar study showed cartilage regeneration when IGF-1 is administered along with the hydrogel composite⁵⁵. A dose-dependent improvement and regeneration was also seen in the study reported by Bessa *et al.* These authors concluded that IGF-1 can promote chondrogenesis by stimulating ECM synthesis and inhibiting matrix-degrading enzymes⁵⁶.
- 6. Platelet-Rich Plasma (PRP):** PRP is a concentrate rich in platelet that is prepared from the whole blood using various centrifugation techniques. It comprises more than 30 GFs, of which 6 GFs are important for cartilage regeneration. It includes TGF- β 1, IGF, FGF, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF)^{57,58}. A study was performed on rabbits that received an IGF-1-hyaluronan mix in PRP in the defects and found cartilage regeneration⁵⁹. An *in vitro* study of sheep chondrocytes propagated in 10% PRP found a significant difference in the number as compared to the chondrocytes cultured in a normal medium⁶⁰. Another clinical study wherein 52 patients had undergone subchondral drilling along with PRP injection showed a better prognosis and cartilage regeneration⁶¹.

V. BIOACTIVE AGENTS

Biologically active substances or bioactive agents can manipulate a living cell/tissue. Several bioactive agents have been studied so far including small and high molecular weight naturally occurring compounds. This chapter will discuss such bioactive agents which have been illustrated for AC regeneration.

- 1. Kartogenin (KGN):** KGN is a small bioactive molecule that can stimulate MSCs differentiation into chondrocytes and was reported by Johnson *et al.* in 2012 for the first time⁶². KGN not only protects the cartilage from degradation but also maintains the chondrocyte phenotype⁶¹. A study of articular cartilage injury in rabbits showed cartilage regeneration post-KGN transplantation⁶³. A similar study in mice reported cartilage regeneration as well as enhanced development of limbs and joints⁶⁴. Recent studies suggested that KGN suppressed OA progression in mice by attenuating inflammation caused by IL-1 β and tumor necrosis factor (TNF- α)⁶⁵. *In vitro* studies suggested that KGN can drastically lower the nitric oxide levels generated by chondrocytes and glycosaminoglycans secreted by cartilage explants validating its protective role⁶¹.

2. **Dexamethasone:** Dexamethasone is a corticosteroid that relieves inflammation and is often used for the management of arthritis caused by cartilage damage. It can promote both osteogenic and chondrogenic differentiation of stem cells. It is often added to the culture differentiation medium and sometimes to hydrogels (covalently bound)⁶⁶. A study reported over-expression of the chondro-related proteins when dexamethasone was added along with TGF- β . The authors found enhanced MSCs and ESCs differentiation to chondrocytes⁶⁷. Dexamethasone is generally employed as a supplement to TGF- β /BMP to stimulate cell proliferation thereby inducing chondrogenesis or osteogenesis⁶⁸.
3. **Simvastatin:** Simvastatin is a medication included in the family of statins that is prescribed to patients with hyperlipidemia and for cardiovascular diseases as an adjunct to diet, exercise, and weight loss. Apart from its lowering effect on low-density lipoprotein (LDL) cholesterol, simvastatin has anti-inflammatory activity that can be utilized for cartilage injury. Animal studies have found significant improvement in collagen-induced arthritis⁶⁹; inhibition of IL-1 β and TNF- α in human chondrocytes in a dose-dependent manner and reduction of nitric oxide (NO) in human chondrocytes⁷⁰. Another study in rabbits demonstrated that localized injection of simvastatin can increase bone-interface healing⁷¹.
4. **Biomaterials for Cartilage Tissue Engineering:** Biomaterials act as a template for tissue regeneration and play a vital role in TE. These materials act as a carrier/scaffold to transfer live cells, GFs, and/or bioactive agents to the injured sites thereby redirecting them to regeneration. Biomaterials are divided into two categories: Synthetic and natural materials.
 - **Synthetic Biomaterials:** Synthetic biomaterials usually are made up of different polymers. Some of them include polyethylene, polyesters, polyurethane, polyvinyl chloride, etc. The most used polymer for AC regeneration is polylactic acid (PLA), polyglycolic acid (PGA), and polylactic-co-glycolic acid (PLGA) which are porous and can carry cells, bioactive substances, and GFs. These polymers possess high durability and good biocompatibility and hence are used clinically for cartilage regeneration in rabbits⁷² and meniscal repair in dogs⁷³. These polymers after disintegration form lactic acid and glycolic acid which are already present in the body and hence are least toxic to the body. Two PLA-based scaffolds have been used clinically for cartilage repair and have shown drastic improvement in OA patients⁷⁴.

Polyethylene glycol (PEG) is a cytocompatible polymer that is used in conjunction with other natural polymers to increase their durability and flexibility in TE. Reports show that PEG-based hydrogel can enhance the chondrogenic differentiation of stem cells in vitro as well as in vivo⁷⁵. Another PEG-HA scaffold could treat full-size cartilage defects clinically⁷⁶.
 - **Natural Polymers:** Natural polymers are always preferred due to their biocompatibility, biodegradability, and least toxicity. Some of the routinely used polymers are agarose, chitosan, hyaluronic acid (HA), fibrin, and collagen.

Agarose is a heteropolysaccharide extracted from red seaweed and has numerous applications. One such application is in cartilage engineering. A study revealed that applying an agarose-based scaffold retained the chondrocyte phenotype and stimulated proteoglycan and GAG levels⁷⁷. The authors encapsulated human chondrocytes in agarose for cartilage regeneration. In another animal study, agarose hydrogel improved the full-thickness cartilage defects⁷⁸. Clinically, an agarose-based scaffold was used for seventeen patients with cartilage defects (over 3 cm²) for over two years and found improved surgical results with hyaline-like cartilage⁷⁹.

Chitosan is a linear polysaccharide obtained from the outer skeleton of shellfish, including crabs and shrimp. The intra-articular injections of the chitosan-hyaluronan hydrogel are effective in repairing cartilage defects due to their structural resemblance to the native cartilage⁸⁰. Another report stated that chitosan hydrogel promotes cartilage regeneration in rabbits⁸¹.

Fibrin hydrogels have been well-documented for cartilage regeneration. A study with fibrin/HA hydrogels containing BMSCs showed enhanced chondrogenesis in vitro confirming its effective role in OA⁸². Another report suggested that chondrocytes can survive in the fibrin gel and can be validated with the increased production of GAG and collagen type II⁸³. As a treatment for cartilage regeneration, Almeida *et al* have used fibrin hydrogel as an injectable containing ECM microparticles that can transform TGF- β ⁸⁴. Due to its efficacy in cartilage regeneration, US Food and drug administration (FDA) has authorized human fibrin hydrogels as a therapeutic agent⁸⁵.

Hyaluronic acid or hyaluronan (HA) is an inherent part of our native hyaline cartilage. Due to its similarity, it is one of the most used biomaterials along with other polymers. A recent study of HA hydrogel combined with PRP in minipigs reported the regeneration of native hyaline-like cartilage after 6 months of the treatment for cartilage defects. The authors also found upregulation in the levels of ECM and type II collagen in the treatment group without the formation of hypertrophic cartilage affirming its use to treat full-thickness cartilage defects⁸⁶. In a study performed on rabbit knees, the application of hyaluronan gel exhibited substantial hyaline cartilage as compared to the control group⁸⁷.

Collagen is an integral protein in the ECM and comprises almost 30% of the total protein mass in the body. Apart from ECM, collagen is also seen in various connective tissues of the body thus making it the largest protein in the body. Due to its integrity, it is used for treating cartilage defects. In a study on rabbit knees, full-thickness AC defects were treated with injectable collagen type II gel⁸⁸. They are often used as cartilage substrates for the treatment of OA⁸⁹.

Although natural polymers lack mechanical stability and flexibility as compared to synthetic polymers, there are ongoing advancements in this field. Researchers are trying to explore many options to come up with the best natural hydrogel for clinical use.

5. 3D Bioprinting Scaffolds: Over the past few years, 3D bioprinting has received massive interest from researchers across the globe for developing functional native cartilage. 3D bioprinting techniques utilize biomaterials, cells, and growth factors as bio-inks which are printed in a predesigned fashion to form a scaffold containing living cells with a specific function for target tissues^{90,91}. Several studies have shown that 3D bioprinting techniques have great potential for cartilage regeneration. Nguyen *et al* used a nanofiber cellulose composite with alginate as bio-ink and printed with human induced pluripotent stem cells (iPSCs) cultured together with human chondrocytes. A cartilage-like construct was developed with cells expressing collagen type II⁹². Studies have also shown successful bioprinting of chondrocytes *in situ* with encouraging results⁹³. Another study utilized 3D bioprinted MSCs along with cellulose/alginate hydrogels *in vivo* in mice. The authors confirmed high mechanical strength and structural integrity post-60 days of implantation⁹⁴.

VI. CONCLUSION

Currently, researchers are focusing on various treatment options for damaged articular cartilage due to its limited healing ability. Conventional surgical treatments focus on reducing the symptoms rather than providing long-term results. Cell-based therapies utilizing stem cells have shown encouraging results, both via experiments and clinically. Many companies have also employed MSCs for bench-to-bedside studies. However, cell-based therapies still have been questioned for the regeneration of hypertrophic chondrocytes over hyaline cartilage. To address the above, more robust clinical validations are required. The rapid advancement in life science, materials science, 3D technology, and engineering has led to progressions in the treatment of various diseases, including cartilage tissue engineering. To date, the primary mode of treatment for OA management is surgical therapies, but soon, we hope to have non-invasive and prophylactic methods as mainstream therapies.

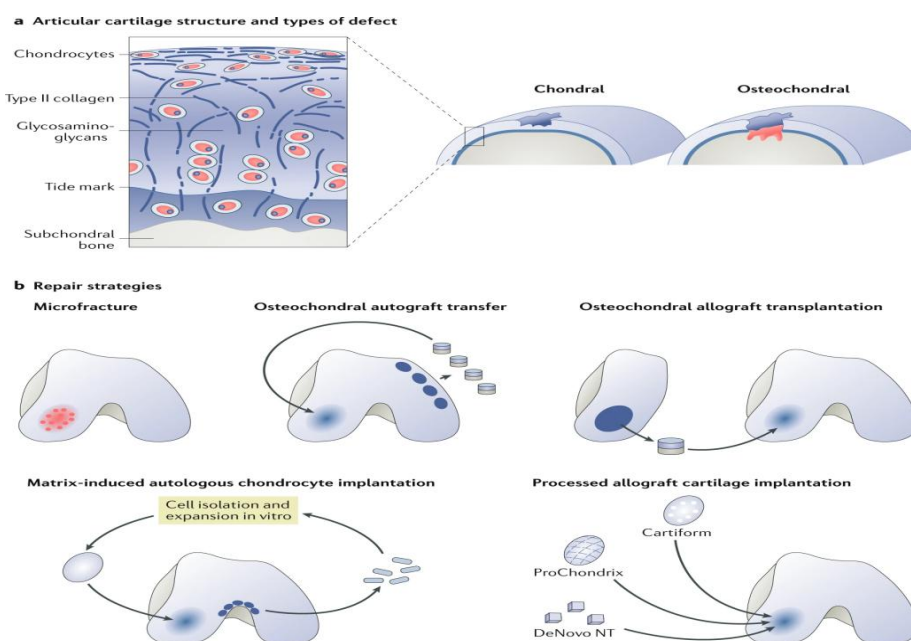


Figure 1: a | Articular cartilage consists of chondrocytes embedded in a defined structure of collagen fibres and glycosaminoglycans. Two main types of defect can occur: chondral defects, which only penetrate the cartilage, and osteochondral defects, which also penetrate the subchondral bone. **b** | Currently used repair strategies for cartilage defects include microfracture, osteochondral autograft transfer, osteochondral allograft transplantation, implantation of processed allograft cartilage such as DeNovo NT, ProChondrix and Cartiform, and matrix-induced autologous chondrocyte implantation. The choice of treatment method depends on the size and type of the defect, the expertise and preferences of the surgeon and patient-specific factors such as age and activity level. (Image adapted from reference [8])

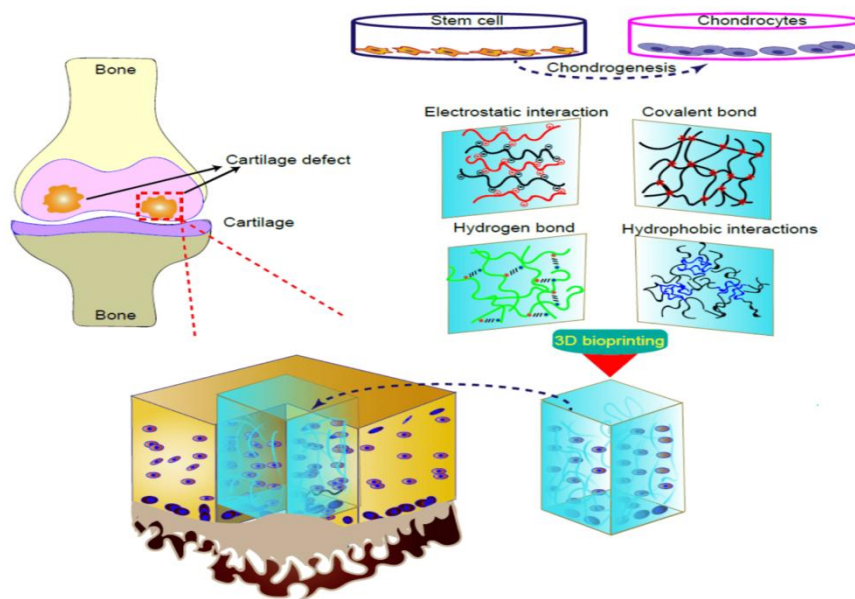


Figure 2. The procedure of creating artificial cartilage tissues with biological functions via 3D bioprinting. The regional characteristics of natural cartilage and the current 3D printing methods used for cartilage defect repair. With the 3D printing technology, the structure is captured from different areas of the cartilage, and the seed collagen fibers are formed into a layered scaffold; they are distributed in ribbons to simulate the structure of natural cartilage.(Image adapted from reference [90])

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