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# Tissue engineering of different cartilage types: a review of different approaches and recent advances

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**Abstract:** Cartilage is a connective tissue that serves as a structural support for maintaining the shape for specific appendices (nose, ear) and also helps for shock absorption when present in joints. Different types of cartilage coexist in the body: hyaline, elastic and fibrocartilage. Due to their different embryologic origin, they produce distinct extracellular matrix and therefore have specific functions according to their location. Cartilage is frequently subjected to many different lesions. Those include traumatic, metabolic and congenital forms, concerning all regions where this tissue is present: joints, head and neck area, intervertebral disks, etc. Increasing number of cancers also affects cartilage; especially in ear, nose and trachea. Unfortunately, this tissue has a poor regeneration ability. Few therapeutic options exist for cartilaginous lesions and most of them concern articular cartilage. They include micro fracture, autologous chondrocytes implantation, mosaicplasty, allograft and prosthesis. Ear and trachea are also targeted for reconstruction with lesser extent. Therefore, cartilage engineering highly addresses increasing number of pathologies associated to this tissue. In the last two decades, several trials were investigated using both progenitor cells and scaffolds. Even bone marrow derived stem cells were widely used and served as gold standard. Many progenitors from different areas are investigated for their capacity of chondrogenesis. On the other hand, biomaterials, natural and synthetic, are used to induce a 3D environment that allows proper growth and differentiation toward cartilage formation. Their characteristics depend on the location of the expected graft where porosity, biodegradability, ability to support strength and large scale use are the key points. Favorable environments are also needed to achieve appropriate chondrogenesis, including biochemical or mechanical stimuli and low oxygen tension. Bioprinting showed also encouraging outcomes in cartilage reconstruction with the investigation of several scaffolds.

**Keywords:** Cartilage;Tissue engineering; Pathophysiology, Therapy.

## Introduction

### Different cartilage types and functions

Cartilage is a connective tissue that serves as a structural support in several areas of the body. Its main function is maintaining the shape (ear, nose) and absorbing shocks (joints). Three different types of cartilage coexist in humans. Hyaline cartilage is the most abundant and is found mainly in joints and also in the trachea and nasal septum. With a blue aspect and a smooth surface, it is usually surrounded by a thin membrane called perichondrium. At biochemical level, the extracellular matrix contains type II collagen, aggrecan, keratan and chondroitin sulfate. Elastic cartilage is found in organs like ear, Eustachian tube, larynx, nostril opening and epiglottis. Its function is to allow both support and flexibility. These characteristics are provided by its high elastin and collagen content. The third type is called fibrocartilage is restricted to areas that need a high resistance to strength, like intervertebral discs, sacroiliac and costochondral joints, or pubis symphysis. In addition to type II collagen, fibrocartilage contains type I collagen as well.

### Cartilage during development

Differences between cartilage composition and functions are explained by their distinct origin during embryologic development. Indeed, hyaline cartilage derives from the mesenchyme with key role of SOX transcription factors during its formation. Under the effect of paracrine factors and hormones, cartilage specific genes and mesenchymal stem cells are activated and differentiated toward chondrocytes (Decker, 2017). The facial cartilage develops from neural crest (neuroectoderm) and mesenchyme. During development, cells migrate to specific locations where they differentiate under the control of *HOX* genes, leading to segmentary organization and different segments in buds of the face and pharyngeal arches (Suzuki and Osumi, 2015). The external ear derives from the first branchial cleft (Anthwal and Thompson, 2016). Tracheal cartilage in its turn, develops from endodermal origin. Beside *SOX* and *HOX* transcription factors, were described to play key roles in cartilage development. Knockdown experiments in *X. laevis* highlighted the role of *FOXN3* in the development of new cartilage. *RUNX3* and *SOX9B* are also involved through a regulation cascade via BMPs (Dalcq,*et al.*, 2012). In addition, *MiR 140* was also described to be involved in cranial cartilage formation via *PDGFRa*, since it provokes cranio facial defects in both zebra fish and mouse.

### Cartilage lesions

Depending on its location, cartilage is subject to several injuries and lesions. In joints, osteoarthritis (OA) and rheumatoid arthritis (RA) are degenerative diseases with multiple causes, including mechanical stresses, genetic factors and trauma. Concerning head and neck cartilage, main lesions are from traumatic, congenital and metabolic origin. Beside, a lot of congenital abnormalities of the ear (microtia, anotia) and the nose (Apert syndrom) lead to heavy and multiple interventions with

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many prostheses or autologous transplantations which could provoke donor sites. The trachea is also subject to trachea bronchial tumors and congenital deformity. Tracheal stenosis is also an especially risk after an intubation. Unfortunately, only few options exist in regards to ear and nose reconstruction, with stents application and tracheotomy. In addition, the growing number of cancers in the area of head and neck affects cartilage and therefore increases the need of its reconstruction in several areas.

**Specificity of cartilage biology**

Cartilage is avascular and non–innervated tissue. During decades, it was described to harbor a single cell type, namely chondrocytes. However, it also contains progenitors which activate and participate in its repair (OA cartilage). However due to its hypoxic microenvironment with a gradient of oxygen comprised between 1 to 7% of oxygen, the regeneration potential is very limited. At biochemical level, the cartilaginous extra cellular matrix (ECM) is specific to the tissue and contains a complex macromolecular network of collagens, proteoglycans and several glycoproteins, and possibly elastin for some areas (ear). The cell–ECM interactions are established through integrins, CD44 and proteoglycans receptors (syndecans). The contents of ECM differs in both quality and quantity, depending on the function and type of cartilage.

**Clinical approaches of cartilage reconstruction**

Historically, OA is the main cartilage disease targeted by reconstruction at clinical level. Therefore, orthopedic surgeons used several techniques with more or less success: microfracture, mosaicplasty, autologous chondrocyte implantation and allograft. Auricular cartilage is also targeted by cartilage reconstruction. Its complex anatomic tridimensional structure is a challenge for reconstruction. Here again, multiple approaches were used between two– and four stage techniques using autologous costal cartilage which led to good results in terms of contour and framework definition. However, long term outcomes show several cons, including the the need to carry out several operations, calcification of the construct, necrosis, contraction of the skin, as well as cartilage resorption (Whatson and Hecht, 2017). Moreover, even if the two–step techniques provides the best pros regarding long term outcomes, limits in its use concern the age of patient concerned (under age of 10) (Jessop et al, 2016). All in all, despite these approaches, it is still yet very difficult to reproduce native tissue with appropriate flexibility, strength and elasticity. Due to its complex anatomy and function, trachea reconstruction undergoes several challenges. It needs to build a safe and stable conduct to ensure breathing without assistance and avoid airways collapses. Therefore, multiple trials were done with insertion of small pieces of cartilage to strengthen the upper airways. The trachea is also more complex since it includes several cell types and tissues, i.e. chondrocytes, epithelial cells and neurons. Recently, assays were done with decellularized matrixes used with chondrocytes and bronchial epithelial cells. Synthetic scaffolds including nanocomposite polymers complexed with growth factors are also tried to optimize progenitor's recruitment in the trachea. The loss of voluminous cartilage substances in head and neck area remains to date without effective treatment in terms of safety, preservation of cartilage properties and aesthetic performance.

**Cells for cartilage tissue engineering**

Like lot of tissue, cartilage undergoes extensive research aiming at its ex vivo reconstruction. They involve the choice of primary cells or progenitors combined with scaffolds, natural or synthetic, as they are decellularized, which are then incubated in appropriate conditions chosen to favor chondrogenesis. Primary chondrocytes would be suitable for this purpose but their small number in biopsies requires extensive amplification. Unfortunately, chondrocytes undergo dedifferentiation during this step, which limits their use in cartilage engineering. In addition, the need of biopsies of cartilage from healthy area is likely to create donor sites. Instead, several progenitors are tested with a lot of advantages, such as the ease of obtaining, several types and tissue sources. The gold standard are mesenchymal stromal cells (MSCs) due to their proliferative rate and multipotency (Baugé and Boumédiene, 2015). Hundreds of studies, in vitro, in vivo or clinical trials, described their suitability to repair large cartilaginous and connective tissue defects. They derived mainly from bone marrow, but are also obtained from blood, umbilical cord, Wharton jelly, amniotic liquid, dental pulp, adipose tissue, synovial tissue and perichondrium (Mazor *et al.*, 2014). Even their molecular signature is not completely defined, these cells are known to harbor several surface markers such as CD28, CD 33, CD44, CD 71, CD73, CD90, CD91, CD 105, CD106, CD 120a, CD 124, CD 131, CD 166 and class I HLA. In addition, MSCs are negative to others markers, mainly hematopoietic ones, i.e. CD14, CD31, CD34, CD45, CD 117. While many tissues contain MSCs, they are not equal for the construction of different types of cartilage. Indeed, specificities of some areas require the expression of peculiar ECM. For example, auricular cartilage which is elastin–rich is more likely obtained by using progenitors from amniotic liquid or from auricular perichondrium (Kunisaki, *et al.* 2007, Takebe, 2012). Therefore, the investigation of multiple sources of progenitors would bring more precise definition on the choice of which cells to use to target a specific cartilage.

**Scaffolds**

A lot of them are assayed for their biocompatibility. Requirements of scaffolds include also immunocompatibility, ability to mimics the host tissue shape. They should also allow cell adhesion, proliferation and differentiation in a tridimensional environment (Korkusuz *et al.*, 2016). Regarding the great variety of tissues to be constructed, their characteristics depend on the location of the graft. Hence, porosity, ability to support strength and biodegradability, are needed for certain tissues. Among natural scaffolds used for cartilage are agarose, alginate, collagens and hyaluronic acid or chitosan (Agrawal and Pramanik, 2019). Beside, several combinations of synthetic biomaterials serve also to mimic the biomechanical properties of cartilage by hosting chondrocytes or differentiating progenitors. The fact that they should be biodegradable is yet a question open to debate. Polyhydroxyacids like PLLA, PCL, PGA and also polyurethan are then used, some of them tried for cartilage reconstruction (Pourbashir *et al.*, 2019). Polyethylen glycol which is already FDA approved is also an option, where its analogs are investigated. Recently, new synthetic scaffolds gave good outcomes. For example, sericin, a silkworm protein that could be functionalized with methacryloyl, and RAD16–I, a self–assembling peptide into nanofiber network yielded promising results in vitro.

**In vitro chondrogenesis environment**

The achievement of chondrogenesis in vitro is a combination of multiple conditions (Ciuffreda, *et al.*, 2016). Addition of growth factors such as *TGFbs*, *IGF*, or *Wnt* as biochemical stimuli are widely used. Indeed, chondrogenic media are used for chondrogenesis induction and phenotype sustaining. In addition, progenitor's differentiation toward chondrocyte may need mechanical tension and sheer stress. CSM are mechanosensitive and then chondrogenesis can be triggered by compression forces which modulate protein synthesis to achieve their differentiation (Gaut and Sugaya, 2015; Glatt *et al.*, 2019). Cartilage is a hypoxic tissue with low oxygen content (1–7%). Therefore, hypoxia is usually used to ensure a proper environment during cartilage engineering. At the molecular level, low oxygen tension activates peculiar transcription factors such as HIF–1 and HIF–2 (Duval *et al.*, 2012), which in turn, modulate multiple target genes. Among them, specific cartilage markers like type II collagen and aggrecan are enhanced in hypoxia, while undesirable ones such as type I collagen are inhibited (Duval *et al.*, 2016). Finally, in vitro chondrogenesis, should include culturing cells in bioreactors. These latter can ensure perfusion of cellularized matrixes, shaking with or without rolling and eventually compression for mechanical stimuli.

**Cartilage bioprinting**

Since cartilage is an avascular and non–innervated tissue, it is obvious that it was one of the first tissues targeted by bioprinting. Indeed, except few progenitors embedded inside, this tissue contains a single cell type, namely chondrocytes. Therefore, it appears simple to try its construction

once 3D printing came to tissue engineering. Several trials were done with different scaffolds or combination of them as bioinks (hydrogels, alginate, gelatin, chondroitin sulfate, lutrol...). They were printed as specific shapes corresponding to particular organs (ear, nose, trachea) before seeding them with cells (Bae *et al.*, 2018; Di Gesu *et al.*, 2019). Therefore, this approach might make bioprinting very beneficial for tissular reconstruction for several reasons. Indeed, it permits to replicate the anatomical forms while reducing surgical techniques and outcomes, to avoid donor site morbidity. However, whatever the methods of biofabrication used, investigations are still ongoing to select the suitable scaffold material to ensure a good encapsulation and printing directly with living cells, knowing that they could be altered during printing and by biomechanical properties of bioinks. Moreover, a good bioink for cartilage should ensure adequate resistance to mechanical strength (Zamborsky *et al.*, 2019).

**Conclusion**

Despite its apparent simple structure with a single cell type, cartilage remains complex to be reconstructed in vitro. Multiple organs that contain this tissue exhibit differences at the biochemical level, explained by divergent embryologic origins and then subsequent roles. These parameters should be taken into account during cartilage engineering by choosing appropriate progenitors that provide the right biochemistry of the desired tissue, and the appropriate scaffold that ensures a good environment for cell proliferation and chondrogenesis. The recent input of 3D printing technology brought encouraging outcomes and will probably help to improve this field by reducing surgical operating times and replicating complex anatomical forms.

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