Section A -Research paper

Application of Placental Tissues Extracellular Matrix in Articular Cartilage Tissue Engineering: A Comprehensive Review

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Abstract

Functional ability is the foundation of healthy aging. A highly structured extracellular matrix comprises type II collagen fibers, proteoglycans, glycosaminoglycans, and other proteins surrounding the chondrons that make up articular cartilage. Articular cartilage acts as a cellular cushion and protects the joints from physio-mechanical stressors. In addition to being one of the most common degenerative disorders that negatively affect the quality of life, articular cartilage deterioration is a major risk factor for osteoarthritis. Owing to the poor self-healing ability of articular cartilage and the less efficiency of existing clinical therapies, regenerative medicine is considered a viable therapeutic option for articular cartilage repair. Effective and enduring long-term results are obtained when articular cartilage defects are treated with autologous chondrocyte implantation (ACI). The last few decades have seen significant advancements in ACI technology, moving from the first generation, which used a periosteal patch, to the second generation, which used a collagen membrane and is currently being replaced by the third generation, which applies chondrocytes within a matrix. Lately, placental tissues have been extensively explored for their contribution to bone and cartilage engineering. The presence of a mixture of extracellular components, and growth factors promotes angiogenesis and reduces inflammation and scarring. All placental tissues have the potential to be useful biomaterials for the creation of novel regenerative therapies due to their compositions and characteristics. Herein, a comprehensive overview of the most recent findings and clinical translation in the context of degenerated articular cartilage, this review article integrates the most significant advancements in tissue engineering techniques including present and future generations of ACI. Comparisons on placental and articular ECM components were also made to explore the use of placental tissues as a biomaterial in bone and cartilage engineering.

Keywords: Autologous chondrocyte implantation, Articular cartilage, Scaffolds, Decellularized cell-derived scaffolds, Placental tissues derived extracellular matrix.

Introduction

Articular cartilage, a kind of hyaline cartilage, covers the articulating surfaces of long bones, the growth plate of the metaphysis, and the sesamoid bones in synovial joints [1, 2]. The porous, viscoelastic composition of cartilage allows for strong load-bearing, and its energy-dissipating lubricating characteristics are partly a result of the matrix's inherent toughness and turgidity, as the internal organization prevents swelling of the tissue [1]. Articular As a cellular cushion, articular cartilage protects the joints from physio-mechanical-physio-mechanical stressors. Mechanical

qualities are provided by the extensive extracellular matrix (ECM) of cartilage, which is maintained by a single group of cells known as chondrocytes [3]. Each chondrocyte in articular cartilage is encircled by a chondron, which is 2-4 m thick and abundant in collagen VI. The initial pericellular environment of the chondrocytes appears to be crucial for regulating cell activity since adult articular cartilage stays avascular [3]. Chondrons are encased in a territorial matrix and a highly organized extracellular matrix (ECM) comprised of collagenous and non-collagenous proteins, proteoglycans, and glycosaminoglycan's [4]. Matrix turnover control and chondrocyte physiology are influenced by a variety of environmental factors, such as matrix composition, soluble mediators (such as cytokines), and biophysical properties brought on by joint mechanical loading. The schematic representation of the articular cartilage structure is shown in Figure 1. The degenerative joint disease causes deprivation, loss, or breakdown of this unique association between the collagenous matrices and proteoglycans, which leads to many complications like arthritis [1]. Knee traumas such as fractures, meniscus tears, and meniscal injury are the primary cause of the degeneration of articular cartilage that characterizes osteoarthritis of the knee. Knee osteoarthritis is diagnosed using a medical history, physical examination, and X-rays. The presence of narrowing joint space on X-rays is critical for diagnosis and eliminates alternative causes of knee discomfort. Pain (mild to severe), stiffness, reduced range of motion in the knee, and localized edema are the most common symptoms. Activity frequently makes knee osteoarthritis pain worse, especially if the affected knee has been overused [5]. Chondrocyte proliferation/clustering and increased productions of aberrant extracellular matrix components are all signs of early osteoarthritis. Excessive catabolic activity is a hallmark of late osteoarthritis, which causes an imbalance in cartilage homeostasis, resulting in aggrecan loss & disintegration [6].

Current treatment Approaches:

Articular cartilage has a low capacity for healing owing to its low cellularity, avascular structure, and predominant type II collagen and glycosaminoglycan composition. The majority of the knee arthroscopies carried out due to injury or symptoms of cartilage degradation were found to have articular cartilage impairment [7]. Current surgical treatments are targeted to repair minor (4 cm^2) cartilage lesions to avoid future degeneration and osteoarthritis progression. Currently, several surgical treatments are being employed in repairing injured articular cartilage and the success rate depends on various factors such as the patient's demographics, the type of injury, any previous surgical procedures, etc [8]. Detailed discussions on the recent tissue engineering approaches are presented in the following sections (i) bone marrow stimulation, (ii) osteochondral allograft and autograft transplantation, (iii) ACI generations, and (iv) future tissue engineering strategies.

Autologous chondrocyte implantation (ACI):

The first human case of autologous chondrocyte implantation (ACI) occurred in 1987, and the pilot study was published in 1994 [9]. ACI is the only available cell therapy now offered by the National Health Service (NHS) in the UK for the treatment of cartilage defects [10].

1st generation ACI:

In the 1st generation of ACI, the wounded area was re-implanted with a monolayer of autologous chondrocytes by an open joint operation, concealed by a natural or synthetic membrane [9, 10]. Due to periosteum-related problems, surgeons stated that this was technically difficult. Few patients experienced post-operative pain at the harvest site due to the additional periosteum that had to be harvested due to periosteal shrinking during surgery [11].

2nd generation ACI:

As autologous chondrocyte implantation (ACI) switched to the 2nd generation, problems with the native periosteum were quickly fixed. Recent technological advances have intended to use cartilage tissue engineering grafts made using 3D matrices that contain autologous chondrocytes for cartilage regeneration to get over the inherent technical difficulties of ACI [12]. Hyaluronan and collagen type I have been employed as biomaterials, and studies on cartilage repair have shown their safety and efficacy. Chondro-glide, a product of geistlich was used to replace the periosteum [13]. Although fewer occurrences of delamination, graft rejection, or the failure of the regenerated tissue to blend with the surrounding native cartilage required re-operation, preliminary investigations with 31 patients demonstrated positive outcomes [14].

3rd generation ACI

In the most recent 3rd generation of ACI, chondrocytes are embedded within scaffolds that have been built in three dimensions to promote cell proliferation. These "all-in-one" grafts can be precisely cut to fit into the cartilage defect using fibrin glue, & they do not require a periosteal cover or fixing stitches [15]. Matrix-autologous chondrocyte implantation or MACI, a commercial product from Germany has reported tackling lesion sizes ranging from 2.3 ± 1 to 11.8 ± 8 cm2. Various independent investigations on MACI have revealed appreciable advancements in terms of clinical results. Despite the benefits of this unique treatment, including the simplicity of surgery, shorter operating times, and the possibility of performing surgery via arthroscopy or a mini-arthrotomy, the product has such a contraindication for use in patients with osteoarthritis [16]. Table 1 explains a variety of commercially available autologous chondrocyte implantation products.

Tissue engineering strategies:

Tissue engineering techniques might offer alternative therapeutic modalities for the regeneration of articular cartilage, as existing surgical treatments don't offer long-term cures. Cell-based technology and extracellular matrix scaffolds will be explained further;

Scaffold-dependent strategies:

Scaffolds have been developed for a variety of purposes in the development of articular cartilage tissue, including cellular differentiation, proliferation, and delivery of chondrogenic bioactive chemicals. For use as scaffolds for constructed articular cartilage, several synthetic and natural materials, including poly-lactides, poly-glycolides, and silk, have been explored. Cell-derived and decellularized tissue cartilages have also been investigated for therapeutic purposes.

Decellularized extracellular matrices:

While preserving the ECM's structural, metabolic, and biomechanical qualities, decellularization techniques aim to remove all the cells and nucleic acids from it. The patient's cells can then be infused back into decellularized tissues and organs to create a customized therapy [24]. Researchers assume that even though decellularized scaffold research and development have advanced significantly, much work still needs to be done to preserve the unique ECM composition, particularly its minor components, evaluate its functionality, and scale up for large tissue and organ replacement. All decellularization techniques continue to be afflicted by substantial issues with immunogenicity, thrombogenicity, and ECM modification [25].

Decellularization of the tissue received from the donor, suitable recellularization, and rebuilding and repair of injured cartilage is the general three phases involved in the cartilage regeneration process using decellularized cartilage scaffolds (DCS). DCS is being investigated more and more as a natural substitute for cartilage repair [26].

Cell-based extracellular matrix:

Another promising tissue engineering technique for facilitating articular cartilage regeneration is the cell-based decellularised ECM (dECM). To use cell-based decellularised scaffolds for cartilage regeneration, it is essential to choose the right cell types to secrete ECM. Numerous types of cells, comprising chondrocytes and several varieties of mesenchymal stem cells (MSCs), have been shown to secrete ECM that resembles cartilage when cultivated in vitro.

To enhance the amount of cartilage-specific ECM released by cells cultivated in vitro within a constrained timescale, specific inducing techniques must be used. There are numerous inducement techniques available at the moment, but the use of specific growth factors is favorable [27, 28]. Growth factors of the TGF superfamily are particularly interesting because they serve essential regulatory functions during cartilage development and it is used widely to stimulate cells to generate more ECM that resembles cartilage. TGF-3 is a key chondrogenic signaling molecule that both promotes and controls chondrocyte growth. Research demonstrates that it improved MSCs' ability to secrete type II collagen and glycosaminoglycans (GAGs) while also inhibiting alkaline phosphatase activity and mineralization, which may help lessen the likelihood of ectopic bone production during chondrogenesis [29, 30].

The chondrogenic capability in terms of matrix secretion and expression of the chondrocyte-specific phenotype are all enhanced by the addition of fibroblast growth factor-2 (FGF-2) to the medium. Apart from this, bone morphogenetic protein-2(BMP-2), and stromal-derived factor-1 (SDF-1) also play a vital role in synthesizing ECM rich in GAGs and Collagen type-II [31, 32].

Mesenchymal stem cells (MSCs) from different sources have been shown to secrete ECM that resembles cartilage when cultivated and induced. After decellularization, the GAG-rich ECM that chondrocytes released into the collagen microspheres helped MSCs differentiate into chondrocytes [33]. In particular, for the therapy of degenerative disorders, mesenchymal stem cells are another promising cell source. Mesenchymal stem cell-derived extracellular matrices were used as an expansion base substance to resuscitate aged mouse-derived mesenchymal stem cells and enhance their capacity for lineage differentiation. It is a biomaterial with good biocompatibility and bio-activity [34]. Moreover, bone marrow-mesenchymal stem (BMSCs) cells have been regarded as another promising cell type due to their distinct multiple differentiation capabilities and quick proliferation and may be collected safely through bone marrow biopsy [35].

Decellularization processes are associated with a few issues. The compositions and structure of the extracellular matrix are indubitably altered by almost all decellularization methods. Additionally, enzymatic and chemical treatments are likely to significantly alter the three-dimensional structure and composition of ECM, whereas physical therapies alone may not eliminate cells and the cell remains. Some researchers try combining physical and chemical treatments to address this issue [36]. Decellularization techniques, which include physical, chemical, and enzymatic treatments, can be broadly categorized into three main groups about the production of decellularised ECM (dECM) to construct scaffolds for articular cartilage repair. Chemical treatments comprising harsh detergents, such as sodium lauryl sulfate and Triton X-100, are administered to disrupt membranes and cause

tissue breakdown and cell lysis, allowing greater penetration succeeding physical treatments. The degradation of proteins and nucleic acids is facilitated by enzymatic processes employing trypsin and nuclease solutions [37]. Figure 2: Diagrammatic representation of methods and decellularization

Cell proliferation and migration are influenced by integrin-mediated cell attachment. ERK1, PI3K, MAPK, and AKT are only a few of the signaling pathways that can be activated by the integrin-ECM mutual effect [38, 39]. The ECM structure might act as a scaffold for the signaling pathways' downstream effector molecules, resulting in more efficient signaling. In line with the known signaling route of the impact of TGF-3 on the chondrogenesis of the mesenchymal tissue, Yang, La [32] discovered that chondrocytes cultivated on bone marrow-derived ECM displayed greater levels of p-SMAD2/3 upon TGF-3-induced chondrogenesis [40]. Therefore, articular cartilage repair in terms of microstructure could be facilitated by cell-based decellularised scaffolds.

Tissue-Derived scaffolds:

Similar to the idea behind using ECM components, the use of tissue-derived materials is founded on the notion that they provide a natural setting for cell migration, seeding, and ECM deposition. These scaffolds can have excellent geometric integrity and bioactivity, but because they must be made from genuine tissue, supply is a concern. The relatively high bioactivity of processed whole-tissue scaffolds has been shown in numerous studies [41].

Sutherland *et al* studied decellularised porcine cartilage for osteochondral tissue engineering [42]. Their study showed that decellularised cartilage outperformed in stimulating chondrogenesis of bone marrow mesenchymal stem cells. They combined physical and chemical decellularisation methods and eliminated around 85% of DNA but at the same time, the levels of glycosaminoglycans were also reduced [42]. The development of an appropriate medium (niche) for regeneration is essential because articular cartilage is a highly specialized tissue with a restricted capacity for regeneration. Due to its abundant ECM content, Özdemir, Emet [43] explored the human placenta, and using a decellularization process, it was changed from being a waste product to a scaffold for cartilage regeneration. They proved that the decellularised placental scaffold was found to be a potential scaffold for the regeneration of hyaline cartilage [43]. Numerous research documents the development of three-dimensional ECM-derived scaffolds by successful decellularization of articular cartilage [44].

Placental tissue-derived ECM:

Another suitable substitute biomaterial for tissue-engineered cartilage is Wharton's jelly ECM. In addition to sharing similarities with articular cartilage-derived ECM, Wharton's jelly ECM (WJECM) also includes a few chondrogenic growth factors. A study reported that both the articular cartilage-derived ECM and WJECM-derived scaffolds were abundant in sGAG and collagens. However, the WJECM had a higher concentration of growth factors than the ACECM, including IGF-I and TGF-, which would be very beneficial for seed cell chondrogenesis. As all the properties were equal in both the ECM Excellent bioactivity and biocompatibility properties were present in the WJECM scaffolds [45].

Human amniotic membrane or hAM, a protective barrier for the fetus is been extensively examined for tissue engineering purposes in articular cartilage repair as it is rich in Growth factors, cytokines, and stem cell-like hAM cells. Additionally, because of the natural structural elements of the ECM like hyaluronic acid, collagens, laminin, fibronectin, and proteoglycans, it has adequate mechanical qualities like permeability, elasticity, and flexibility. After an elective cesarean operation, it is

possible to collect the human amniotic membrane which is extracted from the placenta. It is a biological tissue that is extremely abundant, available, and reasonably priced and that poses no ethical problems. Numerous biological characteristics of hAM have been found to support wound healing. This immune-privileged, biocompatible tissue has anti-inflammatory, anti-fibrotic, and anti-microbial properties [46].

Comparison of extracellular proteins present in cartilage and placental tissues:

Articular Cartilage: Type II collagen makes up the majority of the collagen fibrils in articular cartilage, with minor collagens contributing to the mature matrix's physical characteristics and giving cartilage its tensile strength [47]. Although type VI collagen only accounts for 1% of the total collagen in adult articular cartilage, it is most abundant in the (PCM), which is involved in chondrocyte adhesion and stability [48]. Type IV collagen, laminin, fibronectin, entactin (nidogen); and perlecan, are the main components of basement membranes [49-51]. The multifunctional glycoproteins known as fibronectins are essential for cell attachment. They are heterodimeric molecule that contains I, II, and III types of repetitive domains. Type III repeats make up more than half of the molecule. A single gene can produce many fibronectin molecular isoforms through alternative splicing. These isoforms can identify fibrin, heparin, collagen, integrins, and other cell surface components. Disulfide-linked heterodimers of protein products range in size from 235 to 270 kDa and are embellished with N- and O-linked carbohydrates[52]. Basement membranes frequently contain the prototype laminin molecule, which has α, β, and γ chains. To date, there are 5- α-chains, 3 β-chains, and 3-γ chains, each of which is encoded by a different gene[49]. Another interesting ECM protein present in cartilage is integrins. They are a class of heterodimeric receptor molecules that help cells adhere to the matrix. They maintain mechanical continuity between the inside and outside of the cell. There are currently 18 α and 3 β subunits, which are arranged into 24 different integrins. Integrins are composed of α and β subunits connected by disulfide bonds[53]. A variety of linker proteins, such as vinculin, and paxillin, mediate the inside attachment of integrins to the actin cytoskeleton. A signaling cascade that influences cell proliferation, motility, survival, and gene transcription begins when an ECM ligand engages an integrin[54].

Numerous pericellular matrix (PCM) and ECM proteoglycans, as well as transmembrane Proteoglycans (PG) attached to the chondrocyte cell surface, are found in cartilage. In order for cartilage to function as a weight-bearing tissue, aggrecan, a significant cartilage proteoglycan, and perlecan, a significant pericellular proteoglycan, must imbibe water into the cartilage[55].

Placental Membrane(PM): To maintain the integrity of the barrier in the uterine environment, the placental membrane (PM) is made up of a network of collagens (Collagen type I, type III, type IV, type V, and type VI). I and IV types of collagen are abundant in the chorion, the deepest layer, where they provide scaffolding for placental membrane resident cells. Collagen types I, III, and IV make up the intermediate layer, which also has a higher concentration of proteoglycans and mucopolysaccharides making it attractive to researchers for tissue engineering applications [56, 57]. Glycoproteins, including fibronectin and laminin, are components of the ECM that support the PM's structural integrity. The interaction of amnion and chorionic fibronectin with collagen and proteoglycans impacts cell adhesion, proliferation, migration, and differentiation [58]. Proteoglycans interact or bind with other important ECM components and increase the tensile strength of the membrane which supports cell proliferation and differentiation. It also aids in the binding of growth factors in the other layers of the placental membrane [58, 59]. When used as a biomaterial in tissue engineering, Placental membrane ECM components have been demonstrated to help reduce pain and

scarring at the site of damage, as well as inflammation and infection. Additionally, the ECM has elements that provide the placental membrane elasticity and protection against stress during gestation. Elastin helps the placental membrane maintain its tensile strength and integrity when it expands throughout pregnancy[56]. Another crucial component that gives the ECM flexibility and lubrication is GAGs, such as hyaluronic acid (HA). Studies on the distribution of hyaluronic acid in the placental membrane have found that it is present in parts of the tissue that are rich in collagen, such as the basement membrane (BM) or reticular layer and the intermediate layer of the Placental membrane, but not in the epithelial layer of the amnion's [60, 61]. HA is more prevalent in the chorion and intermediate layers than in the amnion. Additionally, HA has been demonstrated to contribute to the reduction of inflammation and become an attractive component in the field of regenerative medicine [61-63].

Umbilical Cord: The umbilical cord (UC), which connects the placenta and fetus by a tubular structure, serves as a passageway for blood vessels and helps in the transport of oxygen and nutrients to the growing fetus [64]. The honeycomb-like Wharton's Jelly structure is made up of a network of interlinked collagen pores that contain GAGs. The mucoid ground substance is flexible owing to the porous structure when the UC shrinks and grows in response to blood artery contractions. Additionally, these compartments permit the transfer of signaling elements from Wharton's Jelly(WJ) to other regions, like the amniotic cavity[65]. Collagens, proteoglycans, and GAGs, such as hyaluronic acid and heparan sulfate, immobilized and embedded in the collagen network, are the primary components of UC ECM, which is plentiful and maintains the mucoid ground substance[66]. Collagen type I and type III are the most prevalent in this matrix, according to the proportionate relationship of collagen types I (47%), III (40%), V (12%), and other collagens present in the Wharton's Jelly. Their proportionate concentration is also consistent with that of other soft tissues, which usually report having more amount of collagen type I than type III[66]. Proteoglycans including chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratin sulfate also contribute to the structural integrity of WJ by regulating cellular interactions. Since Wharton's Jelly lacks elastin fibers, the GAGs help the substance remain elastic under mild strains and maintain its shape under severe strain [67, 68].

Placental Disc (PD): The placental disc is a temporary organ that enables the exchange of nutrients between the maternal and fetal bloodstreams in order to support fetal development. The placental disc or PD is designed to keep the blood streams apart while optimizing surface area for the delivery of nutrients,in order to establish an immunological barrier Maintaining immune privilege and preventing the maternal immune system from rejecting fetal tissue is one of the placental disc's main roles[69]. Collagens, fibronectin, laminin, proteoglycans, and GAGs play a significant structural role in the placental disc ECM. The distinct collagen type IV is present in the villi basement membranes along with collagen types I and III [70]. Collagen types I, II, III, IV, V, VI, and XIV have also been found in human placental discs. Collagen type XIV has a strong affinity for heparan sulfate, which is derived from the basement membrane, and it may be important *in-situ*[70]. PD is rich in fibronectin and it has been specially studied for its interaction with PGs or proteoglycans. It stimulates cell adhesion, was already proven to accelerate cell migration in vitro, and participates in placental disc organization. [71, 72]. The fibronectin derived from the placenta has a higher resistance to protease degradation and controls how cells connect with tissue and other cells. It can bind twofold to quite so much carbohydrate as the plasma type. Through gestation, the chorionic villi's fibronectin composition changes, and fibronectin is regarded to be important for the formation of the placental tissue [73, 74]. The maternal component of the placental disc, fetal capillaries, and the villous trophoblast all contain laminin. As the villi grow, laminin concentrates more at the mesenchymal cores of the villi where it comes into contact with the trophoblast cell layer [74]. GAGs, such as hyaluronic acid, dermatan sulfate, chondroitin 6-sulfate, trace levels of heparan sulfate, and significant amounts of chondroitin

4-sulfate, have also been discovered to be connected to or related with the collagens of the BM and fibronectin throughout placental disc tissue [75]. In a rat model for full-thickness wounds, placental disc extracts combined with other substances, such as silk fibroin, have demonstrated success. Compared to collagen-incorporated silk fibroin, they demonstrated enhanced angiogenesis, granulation tissue development, and re-epithelialization, resulting in improved epidermal-dermal junctions [76].

CONCLUSION AND FUTURE PERSPECTIVES

Although there have been substantial advancements in the relatively new scientific field of articular cartilage TE (tissue engineering) over the past two decades, there are still many challenges to practical implementation. Translational evaluation is unable to get over this barrier because the design of articular cartilage tissue in the seat-to-bedside procedure lacks translatability and reproducibility. In the realm of cartilage tissue engineering, the one-step, high-quality repair of a full-thickness articular cartilage defect still presents a problem.

Numerous developments in cell sourcing and the utilization of stimuli to create neotissue similar to natural articular cartilage have the potential to offer long-term remedies for cartilage recovery. For instance, the absence of native autologous cells could be compensated for by the use of cells from allogeneic, non-articulating, and/or diseased cartilage, that even though biomimicry is the ultimate goal of tissue engineering, approaches to the field must also focus on producing neotissue that can withstand joint inflammation, easily fuse with its surroundings, and guarantees successful outcomes regardless of biological variability or patient age. Autologous chondrocyte implantation (ACI) and matrix-assisted chondrocyte implantation (MACI), two tissue engineering techniques for articular cartilage repair, have been used in clinics for more than 20 years. On the strength of this achievement, the tissue engineering approach is expanded to osteochondral defect repair, which entails designing articular cartilage, and a smooth cartilage-bone interface. Osteochondral tissue engineering also involves advancing these sophisticated technologies through the FDA because they might include cells and growth agents in addition to biodegradable scaffolds. Scaffold-alone strategies are currently prioritized when it comes to product development because they take regulatory challenges and difficulties into mind.

Healthy extracellular compounds and soluble biochemical components can be found in abundance and variety in placental tissues. Placental tissues are accessible from a screened, healthy population and are otherwise disposed of as medical trash. Examining the complexity of signaling elements that control inflammation, tissue repair and remodelling, in different native placental tissues reveals numerous parallels. The combination of important ECM components including collagenous and non-collagenous substances and growth factors found in placental tissues has consistently demonstrated therapeutic effects in both animal models and clinical investigations. Most of the important ECM components are found to be common in articular cartilage ECM and placental tissue ECM which makes them more attractive for the use of bone and cartilage tissue engineering.

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Table 1: Commercially available autologous chondrocyte implantation products

Generation	**Commercial Product**	**Manufacturer**	**Content of the scaffold**	**Reference**
First Generation	Carticel™	Vericel USA	Autologous chondrocytes	[10]
Second Generation	Chondro-Gide™	Geistlich Switzerland	Refine porcine collagen	[17]
Second Generation	BioSeed-C	BioTissue Technologies Germany	Fibrin, polylactic acid, or polyglycolic polydioxanone	[18]
Third Generation	MACI	Vericel USA	Porcine collagen I/III membrane	[19]
Second Generation	CaRes-1S	Arthro Kinetics Austria	Type-Collagen 1 hydrogel	[20]
Third Generation	Chondrosphere	Co. Don Germany	3D autologous chondrocyte transplantation	[21]
Second Generation	Hyalograft	Anika Therapeutics USA	Benzylic ester of hyaluronic acid (HYAFF)	[22]
Third Generation	NeoCart	Histogenics USA	Bovine collagen type I	[23]
Third Generation	NovoCart	Aesculap biologics USA	Collagen-chondroitin sulfate scaffold	[19]

Table 2: Comparison of important ECM components present in articular cartilage and placental tissues

ECM components	**Distribution in articular cartilage**	**Distribution in placental tissues**
Collagen Type II	Interterritorial Zone and Pericellular matrix[77-79]	Placental Membrane[58]
Fibronectin	Basement membrane[49]	Placental Membrane, Umbilical Cord[58]
Laminin	Basement membrane[49]	Placental Membrane[80]
Collagen Type VI	Territorial Zone[77-79, 81]	Placental membrane and Umbilical cord[58, 82]
Lumican	Chondroadherin[77]	Entire Fetal membrane[83]
Perlecan	Pericellular matrix[84]	Basement membrane of placenta[85]
Decorin	Territorial Zone[84]	Decidua[86]
Aggrecan	Interfibrillar space of articular cartilage ECM[81, 84]	Placental Membrane[58]
Agrin	Basement membrane[81, 84]	Placental Membrane[58]
Fibromodulin	Interterritorial Zone[77, 78]	Lamina Propria of Umbilical tissue [87]
Biglycan	Immediate surrounding of chondrocytes/ Territorial Zone[81]	Umbilical Cord [87]
Collagen Type IV	Pericellular matrix[88]	Placental membrane and Umbilical cord[58, 89]
Collagen XII	Pericellular-Fibril-associated collagens with interrupted triple helices[88]	Placental membrane and Umbilical cord[58, 82]
Matrillin 1	Territorial Zone[77]	NA
Integrin	Basement membrane/Transmembrane receptor[49]	NA
Collagen Type XI	Interterritorial Zone and Pericellular matrix[77-79]	NA
Collagen Type IX	Interterritorial Zone[77-79]	NA
Matrilin 3	Interterritorial Zone[77, 78]	NA
Asporin	Interterritorial Zone[77]	NA

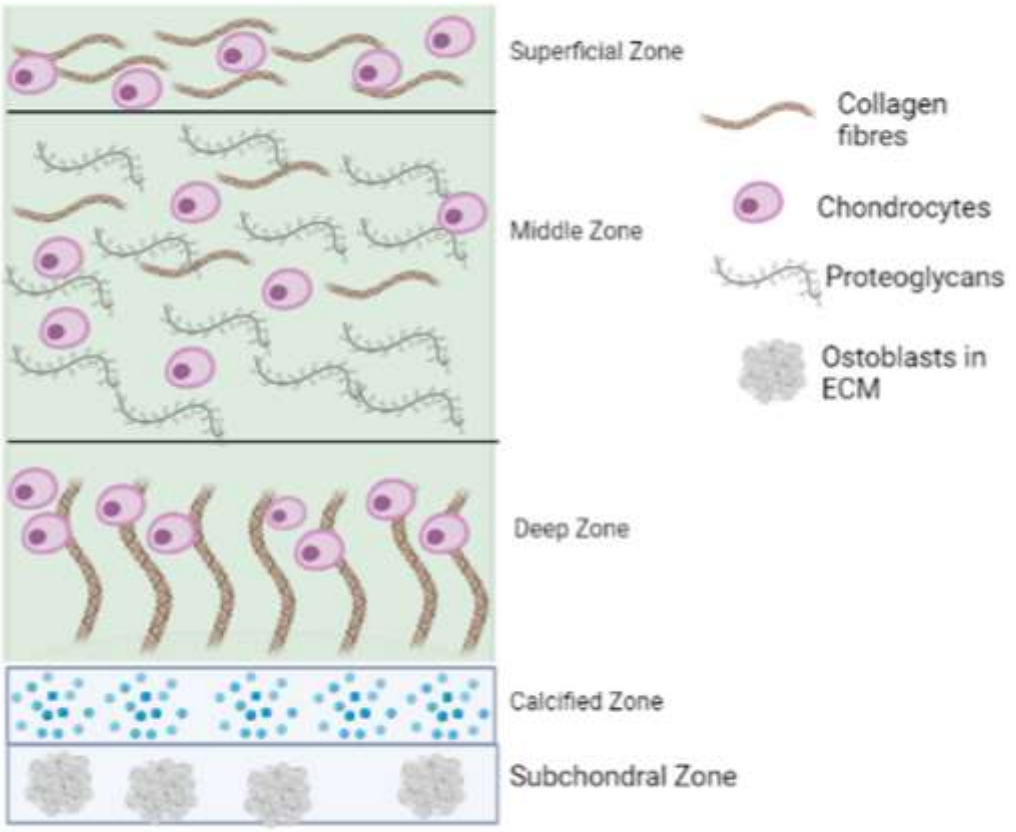

Figure 1: Schematic representation of articular cartilage structure

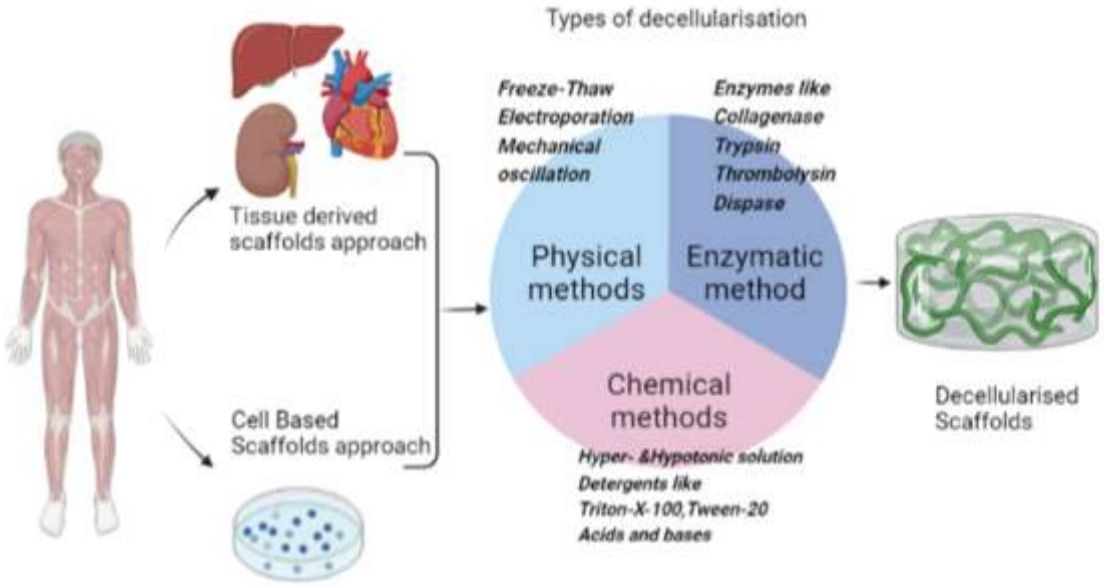

Figure 2: Overview of Decellularisation methods

Table and figure titles and legends:
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TABLE 2: Comparison of important ECM components present in articular cartilage and placental tissues

FIGURE 1: Schematic representation of articular cartilage structure

FIGURE 2: Overview of Decellularisation methods