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SCAFFOLD-BASED TISSUE ENGINEERING STRATEGIES FOR TEMPOROMANDIBULAR JOINT DISC REGENERATION AND REPLACEMENT

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Abstract

The temporomandibular joint (TMJ) disc is a fibrocartilage tissue located between the mandibular condyle and the glenoid eminence, which is central to the TMJ functions. The TMJ disc is susceptible to irreparable degenerative changes or post-traumatic injuries, which can lead to the development of a disc-related disease. Scaffold-based tissue engineering offers the potential for regeneration and replacement of the damaged TMJ disc. The present review describes the biomaterials and manufacturing technologies used in scaffold-based TMJ disc engineering strategies and comprehensively evaluates the advantages and disadvantages of each strategy. As an understanding of the extracellular matrix (ECM) is fundamental for succesful TMJ disc tissue engineering, this review defines the key properties and roles of the TMJ disc ECM. Compared with the natural disc, the mechanical properties of the tissue-engineered TMJ disc are not satisfactory. Additionally, the *in vivo* durability of engineered discs and their long-term impact on the entire TMJ remain to be studied, especially in large-animal preclinical trials.

Keywords: Temporomandibular joint, temporomandibular joint disc, osteoarthritis, tissue engineering, scaffold, regeneration, replacement.

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List of Abbreviations

ASC	adipose stem cell
BMSC	bone marrow stem cell
CSPG	chondroitin sulphate proteoglycan
CTGF	connective tissue growth factor
dECM	decellularised ECM
DPSCs	dental pulp stem cell
DSPG	dermatan sulphate proteoglycan
ECM	extracellular matrix
FDA	Food and Drug Administration
GAG	glycosaminoglycan
hUC	human umbilical cord
hTMSC	human inferior turbinate-tissue-derived MSC
LBL	layer-by-layer
MSC	mesenchymal stem cell
PCL	poly (ε-caprolactone)
PEGDA	poly (ethylene glycol) diacrylate
PGA	polyglycolic acid
PGS	polyglycerol sebacate
PLA	polylactic acid
PLGA	polylactic co-glycolic acid
PLLA	piezoelectric poly (l-lactic acid)
PVA	polyvinyl alcohol
LMP	laser micro-patterning
PTFE	polytetrafluoroethylene
SDS	sodium dodecyl sulphate
TGFβ3	transforming growth factor beta 3
TMD	temporomandibular disorders
TMJ	temporomandibular joint
UBM	urinary bladder matrix
μS	microspheres

Introduction

The TMJ disc is a concavo-convex or saddle-like fibrocartilaginous tissue located between the fossa-eminence of the temporal bone and the mandibular condyle (Detamore and Athanasiou, 2003). It plays a key role in joint biomechanics, especially in transferring loads across the TMJ. The disc protects the underlying condylar cartilage by absorbing loads and lubricating the surface. Many TMJ diseases, including TMJ trauma, ankyloses, osteoarthritis and tumours, are related to TMJ disc destruction or degeneration (Embree *et al.*, 2015; He *et al.*, 2016). As a result of the poor vascularisation, the intrinsic healing capacity of the disc is limited and accelerates the development of disc-related TMJ diseases.

Current management of disc-related TMJ diseases ranges from conservative treatments to surgical intervention procedures. Non-surgical treatments, such as occlusal splints (orthotics) (Al-Moraissi *et al.*, 2020; Luther *et al.*, 2010), analgesics for pain management and behavioural modification, aim to delay the degeneration of mild cases. For some severe cases, the only effective treatment is invasive surgery (Murphy *et al.*, 2013). Surgical options, including disc repositioning or discectomy, have shown efficacy in symptom alleviation (Agerberg and Lundberg, 1971; Hinton, 1992). Unfortunately, without suitable disc substituents, the treatments described above cannot achieve permanent recovery and many patients require follow-up therapy or different surgeries. Total joint arthroplasty is the last option for these patients, leading to a considerable cost burden for individuals and society (Murphy *et al.*, 2013). Thus, there is an urgent clinical need to find an appropriate disc prosthesis to fill the gap in treatment options between non-invasive or minimally invasive strategies and end-stage surgical techniques.

The tissue engineering approach, empowered by the development of materials' fabrication, technological innovation and bioengineering research, has become a promising strategy for regenerating or replacing the fibrocartilage tissue. The scaffold is an essential part of tissue engineering and it provides a framework with desired mechanical properties to help the engineered tissue construct bear the physiological load. In recent years, scaffolds fabricated by synthetic flocculants or natural organic polymers have enabled the reproduction of native fibrocartilage tissue macro- and micro-structure; thus, they can be used as customised implants. This method has been successfully applied in the fabrication of various fibrocartilage tissues, including cartilage (Dadgar *et al.*, 2021), knee meniscus (Li *et al.*, 2020) and intervertebral disc (Ghezelbash *et al.*, 2021). It implies that the use of scaffold-based tissue-engineered TMJ discs will prove to be promising for regenerating or replacing the TMJ disc tissue. Advances in materials and manufacturing technologies have prompted the diversification of TMJ disc tissue engineering strategies, especially concerning the scaffold.

The present review describes the structure and composition of the native TMJ disc, which are crucial references for engineered TMJ discs. The materials and fabrication methods used for the production of scaffolds for TMJ disc tissue engineering are addressed. Furthermore, the barriers in TMJ disc engineering strategies are also discussed.

Biochemical composition and structural properties of the TMJ disc ECM

The TMJ disc consists of a highly hydrated ECM that is mainly composed of collagen and proteoglycans (Fig. 1) (Detamore and Athanasiou, 2003; Detamore *et al.*, 2005; Lowe and Almarza, 2017). The anisotropic collagen structure is essential for the tensile properties of the disc and the many negatively charged GAG side

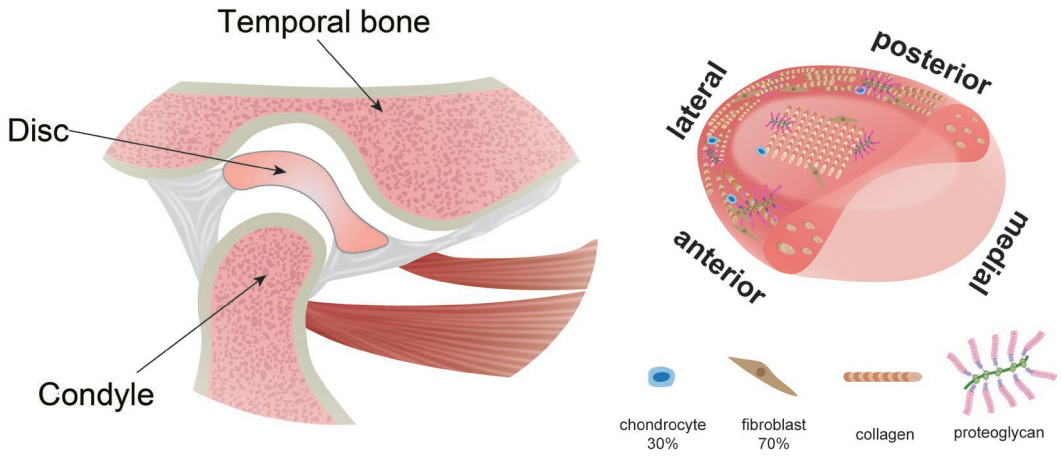

Fig. 1. Biochemical composition of the TMJ disc. The ECM is mainly comprised of collagen type I, chondroitin sulphate proteoglycans and dermatan sulphate proteoglycans. Collagen type I represents 90 % of its collagen content, with other collagens present with quantities less than 10 %, while GAG content ranges from 1 to 10 % in the disc cartilage.

chains of the proteoglycans attract and retain water in the disc, providing its compressive properties.

Collagen accounts for approximately 68.2 % of the disc's dry weight (Almarza *et al.*, 2006) and 90 % of the collagen content is in the form of collagen type I fibres (Almarza *et al.*, 2006). In the antero-posterior direction, collagen content in the intermediate zone is higher than in the anterior and posterior zones. In the mediolateral direction, higher collagen content is found in the central region compared with the lateral region (Almarza *et al.*, 2006).

The GAG content of the disc ranges from 1 to 10 % in the disc cartilage, depending on the region (Detamore *et al.*, 2005). Chondroitin sulphate and dermatan sulphate GAGs are the most abundant GAGs found in normal human discs, comprising 90 % of the total GAG in the anterior-middle zone and 84 % of the total GAG in the posterior zone (Axelsson, 1993; Axelsson *et al.*, 1992; Okazaki *et al.*, 1996). In addition, hyaluronic acid accounts for approximately 5 to 20 % of the total GAG content (Axelsson, 1993; Axelsson *et al.*, 1992; Okazaki *et al.*, 1996). Keratan sulphate and heparan sulphate GAGs are also present but in trace amounts (Axelsson, 1993; Axelsson *et al.*, 1992; Okazaki *et al.*, 1996). Except for hyaluronan, all these GAG chains are covalently attached to protein cores of the diverse family of proteoglycans. Both versican and aggrecan, two of the so-called large proteoglycan sub-family that non-covalently bind to hyaluronan to form large aggregates, are found in the disc. They have a high molecular weight (> 10^6 Da) and their attached GAG side chains are mainly chondroitin-sulphate-6 (Nakano and Scott, 1989). These CSPGs are present in higher concentration in the central part of the bovine disc than in the peripheral regions (Kuc and Scott, 1994). In rat discs, CSPGs were abundant at the junction between the anterior zone and the attachment (Mizoguchi *et al.*, 1998). DSPGs are a group of small proteoglycans, including biglycan (DS-PGI) and decorin (DS-PGII) (Kuc and Scott, 1997; Scott *et al.*, 1995), which are present in the disc. Decorin is mainly found at the junction between the disc and the attachment and is more abundant in the peripheral zone than in the central zone (Mizoguchi *et al.*, 1998).

Structure properties of the ECM

In the disc intermediate zone, the collagen network is arranged predominantly in the antero-posterior orientation. The highest density of collagen fibres is observed in the medial part, enabling the disc to withstand physiological stress during jaw movement. In the peripheral zone, the collagen is aligned around the central zone of the disc in a ring-like pattern. These structural features suggest that collagen fibres may act as a buffer of tensile force. In accordance with the regionally anisotropic structure of collagen, regional variation in the TMJ disc mechanical properties has also been reported (Berkovitz and Robertshaw, 1993; Minarelli *et al.*, 1997). In the antero-posterior direction, the stretch modulus of the central zone in the TMJ disc is much lower than in the anterior and posterior bands (Teng *et al.*, 1991). In the mediolateral directions, the compression modulus of the central part is 2-3 times larger than in its periphery (Beek *et al.*, 2001). Additionally, the anisotropic collagen fibres intercalate with each other, presenting a net-like structure with porosity. Zhang *et al.* (2014) discovered that the range of pores of 150-250 μm is beneficial for cell growth and nutrient infiltration, increasing the formation of the cartilage. Based on the characteristics of this structure, the biomimetic anisotropic scaffold fibre arrangement is of great significance in achieving good mechanical properties and successful tissue regeneration for disc tissue engineering.

Current studies on the biochemical composition of TMJ discs have revealed the weight ratio of collagen and GAG in the total TMJ disc. However, the accurate biochemical composition based on the component partitioning and its influence on the function of the TMJ disc is rarely reported. Studies have revealed that a ring-shaped structure surrounded the antero-posterior arrangement of collagen; however, there is a gap in knowledge about TMJ disc micro-structure, such as the unexplored spatial heterogeneity of the collagen's orientation and how it contributes to the function of the articular disc. To build an engineered scaffold close to the native disc, a high-resolution analysis of the ECM structure at the micro- and nano-level is needed to allow for the replication of the structure.

The role of mechanical loading

The TMJ disc is an essential protective fibrocartilage. Discs withstand tensile, compressive and shear forces during jaw movement. Its mechanical strength is vital for absorbing load, reducing friction and protecting the condylar surface. Many diseases can destroy or degenerate the TMJ disc's mechanical function. When the TMJ disc bears long-term stress that exceeds the stress-limit that the site can withstand, irreversible damage will occur.

The tensile properties of the TMJ disc are mainly related to the direction and arrangement angle of the collagen fibres (Allen and Athanasiou, 2006). The tension process of the TMJ disc can be divided into two stages. The first stage is the elastic deformation stage, during which the external force is removed and the TMJ disc can be completely restored to its original length (Shengyi and Xu, 1991). The second stage is plastic deformation, when the external force cannot be restored to its actual length. The TMJ disc will be destroyed once the external pressure exceeds its ultimate strength. Its compression performance is related to the distribution of GAG and regions with high GAG content can be expected to have a higher relaxation compression modulus. The shear stress experienced by the TMJ disc is mainly manifested as the relative displacement between the inferior and superior layers. The shear modulus of the TMJ

disc can be one tenth of its compression modulus (Barrientos *et al.*, 2019). Tissue damage may be mainly related to long-term repeated tensile and shear stress (Iatridis and ap Gwynn, 2004).

Scaffold materials for tissue engineering of the TMJ disc

An ideal tissue-engineered disc scaffold should have the following characteristics: 1) good biocompatibility to meet *in vivo* application; 2) biological microenvironments favourable for cells to synthesise the TMJ disc ECM; 3) suitable degradation rate matching the tissue formation; 4) long-term functional stabilisation to bear the constant and heterogeneous mechanical loading. To meet these criteria, viscoelastic properties, appropriate mechanics, tissue anisotropy, macro geometry and surface lubrication should be considered in the design. The present study reviewed the materials used in scaffold-based repair and replacement of TMJ disc (summarised in Table 1**a**,**b**) and summarised the scaffold materials used for tissue engineering of the TMJ disc in four categories according to their source and chemical characteristics: synthetic polymers, natural materials, hydrogels and dECM scaffolds.

Synthetic polymer scaffolds

Synthetic polymer materials allow the detailed shaping and tailoring of mechanical properties with the aid of advanced manufacturing technology. Polymers, including PTFE (Springer *et al.*, 2001), PCL (Legemate *et al.*, 2016; Moura *et al.*, 2020; Tarafder *et al.*, 2016), PGS (Hagandora *et al.*, 2013), PLA (Ahtiainen *et al.*, 2013), PGA (Almarza and Athanasiou, 2004; Almarza and Athanasiou, 2006; Detamore and Athanasiou, 2005), PLLA (Liu *et al.*, 2022) and PLGA (Tarafder *et al.*, 2016), have been investigated for the construction of the TMJ disc. Researchers have attempted to utilise Proplast-Teflon disc implants for TMJ reconstruction after discectomy (Estabrooks *et al.*, 1990; Henry and Wolford, 1993). The implanted materials have poor mechanical properties and are incapable of bearing the load under functional movement. In addition, debris from these synthetic materials was found to induce more severe complications, such as foreign-body reactions, condylar absorption and osteoarthritis (Vincent *et al.*, 2016). These results motivated researchers to develop TMJ disc substitutes with better biocompatibility. Hagandora *et al.* (2013) have inoculated goat fibrochondrocytes into porous PGS, which successfully induced matrix production. However, according to Almarza and Athanasiou's research (2004), the degradation rate of the PGA scaffold seeded with porcine TMJ disc cells exceeds the rate of new matrix formation, leading to shrinkage of the scaffold, which affects the mechanical properties of the whole construct in the process of new matrix synthesis (Almarza and Athanasiou, 2004). Various efforts, such as using polymers with a low degradation rate as that of the scaffold, were made to coordinate matrix generation and scaffold degradation rate. Ahtiainen *et al.* (2013) reported tissue-engineered non-woven PLA discs seeded with autologous ASCs as a potential replacement for the TMJ disc. A large number of PLA fibres were still observed by 12 months. However, it was hard to determine whether the mechanical properties of the construction were provided by the new matrix or the residual PLA material.

More recently, research with synthetic polymers has been aimed at producing biomimetic and bioactive scaffolds that closely mimic the anisotropy of the TMJ disc. 3D printed scaffolds, modified by growth factors conducive to producing anisotropic and regionally heterogeneous fibrocartilages, were fabricated. CTGF and TGFβ3 were encapsulated into PLGA microspheres in the PCL scaffold and distributed in an accurate spatiotemporal way. MSCs were incubated on the scaffold and successfully induced to secrete native TMJ disc-like heterogeneous fibrocartilage matrix in 6 weeks (Legemate *et al.*, 2016). The regenerated construct showed improved biocompatibility. In addition, the scaffold resulted in compressive modulus, relaxation modulus and coefficient of viscosity that were close to that of the native tissue. However, the performance of this scaffold in long-term *in vivo* follow-up studies needs to be evaluated.

Despite the advancements described above, achieving appropriate cell distribution in synthetic polymer scaffolds remains a challenge. Poor initial anisotropic cell attachment and uneven cell distribution in the large construct would hamper the regeneration effect. In addition, maintenance of the scaffold's mechanical properties remains a challenge because the degradation rate of synthetic polymer materials does not match the rate of new matrix generation. However, a recent scaffold-based strategy has introduced new ideas for tissue-engineering of TMJ discs. Liu *et al.* (2022) used piezoelectric PLLA nanofibre scaffolds to stimulate cartilage regeneration. Studies have demonstrated that piezoelectric scaffolds can be used as a biodegradable stimulant by generating surface charges that are beneficial for cartilage growth under controlled mechanical stimulation (joint movement).

Naturally derived scaffolds

Naturally derived materials are macromolecules abundant in native tissue, including collagen, fibrin, alginate and chitosan. Collagen is a predominant component of the native disc and has gained attention due to its porous structure that is favourable for cell growth and GAG infiltration. In terms of tissue engineering of the TMJ disc, Thomas *et al.* (1991) attempted to apply collagen I mesh to engineer the ECM of the TMJ disc. However, the extracted collagen fibres were loose and weak in structure. To make the fibres more robust mechanically, thermal crosslinking

Table 1a. Different types of scaffolds and fabrication methods used in TMJ disc tissue engineering.

Different types of scaffolds used in TMJ disc engineering		Fabrication technique	Experimental model	Mechanical analysis	Main outcome
Synthetic polymer scaffolds	PCL, PLA, PGA, PLGA μS	PCL fibre embedding with PLGA μS encapsulated with CTGF and TGFβ3 μS, 3D printed	*In vitro*, hMSCs, 6 weeks *In vivo*, New Zealand white rabbits, 4 weeks	None	Anisotropic collagen orientation Increase in collagen synthesis within perforated defect compared to empty μS-embedded scaffolds
	PCL	3D-printed PCL scaffold with spatially embedding CTGF- or TGFβ3-encapsulated μS (100 mg μS/g of scaffold)	*In vitro*, hMSCs, 6 weeks	Tensile modulus, compressive modulus	Regionally variant matrix synthesis (collagen type I and II) Region-dependent viscoelastic properties
	PLGA	Electrospun membranes	*In vitro*, TMJ disc cells and rabbit synovium-derived MSCs, 2 weeks	None	The electrospun PLGA scaffold-loaded cell sheets could form an articular disc tissue with certain morphological characteristics
	PGS	-	*In vitro*, goat fibrochondrocytes, 4 weeks	Compression tangent modulus	PGS is biocompatible, biodegradable and porous Abundance in both collagen and GAG in PGS scaffolds seeded with 50 and 100 million cells/mL goat fibrochondrocytes compared with 25 million cells/mL
	PLA	ASCs were incubated on PLA	*In vivo*, rabbits, 6 and 12 months	None	PLA discs were visible 6 and 12 months after implantation No signs of infection, inflammation or foreign-body reactions
Naturally derived scaffolds	Fibrin, chitosan	Fibrinogen solution was dropped onto chitosan scaffold, followed by adding thrombin solution to form fibrin/ chitosan scaffolds	*In vitro*, TMJ-SDSCs, 4 weeks *In vivo*, nude mice, 4 weeks	None	Cartilage ECM deposition in fibrin/ chitosan scaffold TMJ-SDSCs to regenerate fibrocartilage
	Alginate, chitosan	Chitosan/alginate scaffolds were fabricated by crosslinking with $CaCl_2$ combined or not with glutaraldehyde	*In vitro*, DPSCs, 8 weeks	None	DPSCs incubated on chitosan/ alginate scaffolds with abundant fibrocartilaginous ECM deposition

Table 1b. Different types of scaffolds and fabrication methods used in TMJ disc tissue engineering.

Different types of scaffolds used in TMJ disc engineering		Fabrication technique	Experimental model	Mechanical analysis	Main outcome
Hydrogel scaffolds	PEGDA hydrogel, PCL	The PEGDA hydrogel was injected into the PCL scaffolds	None	Compressive modulus 13.53 ± 1.30 MPa	Multi-material scaffolds with hydrophobic nature, porosity Compressive modulus closer to the native disc
	PVA, PCL	Printed PCL scaffolds were infused with PVA hydrogel solution	*In vitro*, rabbits' chondrocytes and fibroblasts *In vivo*, goats, 12 weeks	Compressive modulus 10.199 ± 0.984 MPa, cyclic compressive tests, creep curve, tensile tests, cyclic tensile tests	An innovative way to induce disc-defect repair for 12 weeks in goats; mechanical strength similar to that of the natural disc
dECM scaffolds	UBM	Decellularised porcine urinary bladder tissue scaffold (UBM)	*In vivo*, mongrel dogs, 6 months	Compressive mechanical properties	Similar to those of the native TMJ disc from gross morphological, histological, biochemical and biomechanical perspectives
	ECM of TMJ disc	Laser micro-patterning: CO_2 laser ablation system to drill micro-pores within porcine TMJ discs	*In vitro*, hMSC	Compressive modulus 2.20 ± 0.24 MPa	Enhanced cellular infiltration, while not significantly altering biochemical and biomechanical properties of the native tissue
	ECM of TMJ disc	Laser micro-ablation: CO_2 laser ablation technique has been used to generate artificial micro-pores within porcine TMJ discs	None	-	Laser ablated channels incorporated after SDS treatment were relatively smaller and more uniform, indicating surfactant pre-treatment is an important consideration when using LMA to produce artificial pores

was performed to treat collagen fibres and autologous BMSCs were inoculated on the fibres (Kobayashi *et al.*, 2015). In the rabbit perforation model, the defect regenerated by the collagen sponge scaffold with BMSCs was completely repaired after 2 weeks (Kobayashi *et al.*, 2015). Nevertheless, there is no biomechanical evidence to assess the effectiveness of TMJ disc regeneration on function. So, its application to large-size tissue engineering constructs needs to be further investigated.

Both fibrin and chitosan are suitable materials for fibrocartilage tissue engineering. Fibrin, which favours cell proliferation and ECM deposition, has been approved by the FDA for clinical use. However, fibrin alone is inadequate to engineer the TMJ disc due to its poor mechanical strength, rapid degradation and volume shrinkage (Li *et al.*, 2015). A hybrid scaffold of fibrin gel and lyophilised chitosan has been investigated with TMJ-derived synovial stem cell cultures. Then, the chondrogenically induced construct was implanted into nude mice. After 4 weeks, more cartilage ECM deposition was observed on the fibrin/chitosan scaffold than on pure chitosan; yet data on biomechanical performance were not presented (Wu *et al.*, 2014b).

Another naturally derived material frequently used in scaffolds is alginate. Alginate is a hydrophilic, non-toxic, biocompatible and non-antigenic anionic polymer abundant in seaweed and has been extensively applied in cartilage tissue engineering (Popa *et al.*, 2015). A combination of alginate and chitosan through cross-linking can produce a bionic (in structure and composition) joint-disc scaffold. For instance, a chitosan/alginate scaffold crosslinked by $CaCl_2$ promotes biological behaviour of cells and induces growth of fibrocartilage tissue similar to that

of the native TMJ disc (Bousnaki *et al.*, 2018).

The significant advantage of naturally derived materials is their excellent biocompatibility, reflected in the promotion of cell adhesion as well as differentiation and deposition of new ECM. However, the intrinsic characteristics of these components, such as poor mechanical strength and fast degradation, make them less optimal for engineering TMJ discs. Combining these materials with other materials is one possibility for improving the properties of the naturally derived scaffold.

Hydrogel scaffolds

A hydrogel is a hydrophilic colloid with a three-dimensional network structure. It swells rapidly in water and holds a large volume of water without dissolving (Lee and Kim, 2018). Similarly, the native TMJ disc is also characterised by a high water content and can buffer and absorb shock when loading occurs during jaw movement, a property defined as viscoelasticity. As reported, hydrogels can be created that have distinctive mechanical properties, such as low friction and viscoelasticity, that match the cartilage tissue (Hua *et al.*, 2021). Combinations of natural materials or artificial polymers, or both, can be used to generate hydrogels with mechanical properties closer to that of the native TMJ disc. For example, a combination of PCL with a PEGDA shell produces a hydrogel with the geometry and compressive modulus of the ovine TMJ disc (Ângelo *et al.*, 2021; Moura *et al.*, 2020). In another study, the porcine acellular matrix of the TMJ disc was processed to a powder and treated chemically to make a hydrogel. *In vitro*, costal chondrocytes infiltrated the hydrogel, showing good cell differentiation in culture (Liang *et al.*, 2020). The hydrogel was injected subcutaneously into mice and initial results indicated good cell survival and morphology, with angiogenesis occurring at the periphery. However, the hydrogel degraded after only 7 d *in vivo*, which might be attributed to its low concentration in the ECM (Yang *et al.*, 2017).

Hydrogels are widely used in cartilage tissue engineering due to their viscoelasticity, similar to the characteristics of natural TMJ discs. Theoretically, the introduction of ECM with a similar biochemical composition to native disc could be beneficial for achieving the desired mechanical properties and excellent biocompatibility.

dECM scaffolds

Decellularisation is a process (Fig. 2) in which cellular components are removed from the tissue to reduce immunogenicity, preserving the ultrastructure of the original tissue and important functional proteins (Hynes, 2009). This technique is widely used in tissue engineering, including for heart, lung, kidney and liver. Several methods are applied for producing acellular scaffolds, depending on the tissue used. Physical decellularisation methods include multiple freeze-thaw cycles, agitation during immersion in a chemical solution and application of pressure, all of which aim to destroy the structure of cells and remove the nucleic acid inside (Agmon and Christman, 2016; Badylak *et al.*, 2009). Enzymatic decellularisation, using nucleases, collagenases and proteases, acts on the connections between the cell and ECM or networks among cells (Lynch and Ahearne, 2013). Chemical decellularisation detergents, including SDS and Triton X-100, degrade the cross-links of the fibrous components and proteins of the matrix, which is conducive to the removal of cellular components from the matrix (Keane *et al.*, 2015). Decellularisation methods can result in less than 50 ng/mg dsDNA (dry weight), < 200 bp DNA fragment length (Nagata *et al.*, 2010) and undetectable nuclear components on histological staining (Crapo *et al.*, 2011).

Theoretically, the dECM scaffold can retain the structure and biochemical components of the ECM, including the anisotropic collagen microstructure, all of which are essential for the mechanical strength of the construct and the structural support of cells. Two

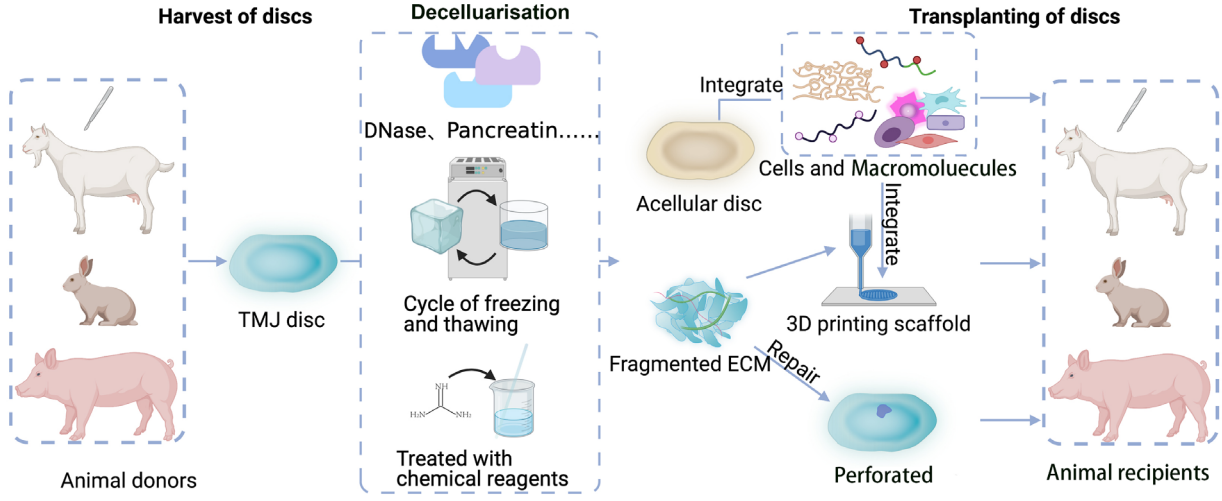

Fig. 2. The procedure of decellularisation of the TMJ disc and subsequent application. After TMJ discs are harvested, decellularisation is completed through enzymatic, physical and chemical treatments. The decellularised TMJ disc can be used as a material in tissue engineering.

strategies have been attempted, utilising the entire macro structure of decellularised TMJ disc or the extracted dECM to regenerate a new matrix.

The dECM is an ideal scaffold due to its good biocompatibility and superior biomechanical properties. However, dECM scaffolds are often limited by low porosity, hindering cellular infiltration and uniform regeneration. Utilising a technique named LMP, it is possible to create microporous structures on the acellular matrix, facilitating cell ingrowth into the scaffold (Juran *et al.*, 2015). Multiple studies have confirmed that the porous structure and appropriate pore size are critical for the function of cells and the regeneration of new matrices (Matuska and McFetridge, 2018; Wu *et al.*, 2014a). Over-large pore size will lead to rapid degradation of collagen fibres.

A small pore size affects cell infiltration and their deposition of new matrix. Hence, the appropriate micropore diameter (120-300 μm) is essential for maintaining of biomechanical properties and promoting appropriate cell function (Matuska and McFetridge, 2018). Juran *et al.* (2015) used a CO_2 laser to construct a uniform and homogeneously distributed micropore structure in an acellular matrix with a micropore diameter of 120 μm and inoculated MSCs isolated from the hUC into the scaffold. The cell density in the LMP group was significantly higher than in the non-LMP group and the cells had higher metabolic activity. Moreover, compared to the non-LMP scaffold, where the hMSCs were mainly located on the surface layer of the scaffold, the engineered micropores of LMP were occupied by cells throughout the entire thickness of the construct and distributed evenly. The LMP scaffold exhibits a compression modulus close to that of the native TMJ disc (Juran *et al.*, 2015). However, the scaffold has not been evaluated in *in vivo* experiments. The degradation rate of the microporous structure of the acellular matrix, changes in pore size during degradation and comprehensive mechanical properties under loading need to be assessed.

As an alternative to modifying the intact dECM tissue scaffolds to replace the damaged disc, dECM can also be powdered to produce bioactive particles. One study used porcine dECM powder harvested from urinary bladders, encapsulated by hydrated sheets of UBM, to regenerate TMJ disc in a canine discectomy model (Brown *et al.*, 2012). After 6 months, the shape of the pillow-like scaffold resembled that of the native TMJ disc and the content of collagen and GAG in the scaffold was close to that of the native disc. Immunohistochemistry results revealed no $CD68^+$ macrophages in the tissue. Furthermore, small blood vessels similar to that of the native TMJ were also observed in the new tissue, indicating regeneration in this construct. In terms of mechanical performance, the reconstructed pillow-like scaffold displayed a compression modulus similar to that of the native disc (Brown *et al.*, 2012). This study described a new strategy for the tissue engineering of TMJ discs.

The dECM-scaffold-based strategy can retain part of the basic structure of the TMJ disc, with most of the ECM biochemical components, and provide the cells with the microenvironment closest to that of the original joint disc. For dECM scaffold preparation, other fibrous cartilages, such as intervertebral disc or meniscus could be considered.

Several materials have been used for TMJ disc regeneration or replacement. Each material has its unique advantages and disadvantages. Researchers must consider the advantages and disadvantages of various materials to create combinations with superior properties.

Advanced fabrication techniques for the construction of TMJ disc scaffolds

The emergence of new fabrication technologies, such as decellularisation, 3D printing and LBL nanoassembly, provides new avenues for the construction of TMJ scaffolds.

3D printing

3D printing, a component of additive manufacturing, is a computer-controlled process for constructing a designed 3D body layer by layer and is widely used in fibrocartilage tissue engineering. 3D printing methods can be sorted into inkjet, extrusion and laser-assisted printing. The inks for printing can be classified into biomaterial inks (cell free) and bio-inks (cell laden). Synthetic or natural polymer scaffolds built by 3D printing can be customised to the regionally variant microstructure (Legemate *et al.*, 2016; Tarafder *et al.*, 2016). They can resemble the size and shape of the native TMJ disc at the micro-scale and show good compressive and region-variant anisotropic tensile properties. The three-dimensional spatial structure also provides the space for cells' growth and guided matrix formation.

When tissue engineering fibrous cartilage using bio inks, some researchers used dECM with hASCs and hTMSCs as the bio-ink and applied 3D printing technology to customise the microstructure of the target tissue with high resolution and hierarchical organisation (Vernengo *et al.*, 2020; Pati *et al.*, 2014; Visscher *et al.*, 2021). These constructions can optimise the microenvironment and facilitate the formation of three-dimensional structured tissue, which is conducive to reconstituting the intrinsic cellular morphologies and functions (Pati *et al.*, 2014).

LBL nanoassembly

LBL nanoassembly is a fabrication method utilising the principle of electrostatic attraction between dissimilar charges to assemble nano-films layer by layer. Nano-structured modification may influence surface wettability, free energy and surface roughness

of the materials, which are conducive to the adhesion of cells and maintenance of the cellular phenotype (Kommireddy *et al.*, 2006; Torrecillas *et al.*, 2009). On surfaces modified by LBL nanoassembly, the attachment of TMJ discal fibrochondrocytes and the synthesised ECM increases as the thickness of the nanomembrane increases. The content of type I collagen and proteoglycan in the newly formed matrix is similar to that of the TMJ disc tissue (Ronald and Mills, 2016). The principle of LBL nanoassembly can be applied to materials with poor surface adsorption, which can help the adhesion and differentiation of the cells to broaden the range of materials to choose from.

Concluding remarks

The development of biomaterials and technical innovations have contributed significantly to scaffold-based tissue engineering for the TMJ disc. However, although many tissue-engineered scaffolds have been tested, none have resulted in effective TMJ disc regeneration or have been a satisfactory replacement tissue. There are still many issues that need to be resolved before the clinical application of any tissue-engineered TMJ disc implant.

An in-depth understanding of TMJ disc micro-composition and micro-structure is the backbone for tissue engineering approaches. Studies have previously shown the native tissue structure-function relationship. Since the anisotropy of the TMJ disc provides the regional mechanical properties, a poor understanding of the complex native disc construct is still a remaining hurdle for TMJ disc tissue engineering. More detailed investigations are needed to define the composition and the micro-structure of the TMJ disc.

As stated above, the mechanical properties of tissue-engineered TMJ discs are not yet satisfactory compared to the native disc. The mechanical properties are the essential characteristic of the TMJ disc, which functions under dynamic stresses, including compression, tension and shear. Existing tissue-engineered TMJ disc scaffolds cannot meet all aspects of the mechanical demands. This requires extended biomaterial research and continuous evolution in techniques that promote improvements to the mechanical property of tissue-engineered TMJ discs.

Several studies have demonstrated that the engineered TMJ disc is lubricative and smooth enough for TMJ movement and can prevent degenerative changes in the condyle. However, the *in vivo* durability of the engineered disc and the long-term effect on the whole TMJ remains to be studied. Verifying the long-term condyle-protected function and longstanding mechanical performance of engineered discs *in vivo*, especially in experimental large-animal studies, will be a necessary step for translating these engineered discs into a human medicine.

Scaffold-based tissue engineering research could lead to the development of innovative and effective treatments to repair or replace the injured TMJ disc.

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Discussion with Reviewers

Reviewer: Although the tissue-engineered constructs have got more sophisticated, they remain inadequate for TMJ replacement. Have the authors considered that the underlying pathology (degenerative disc disease) may not be addressed with only providing a new disc?

Authors: Management of disc-related TMDs varies with disease severity. Non-invasive and minimally invasive strategies include physical therapy, occlusal splints or adjustments, pharmacological agents, sodium hyaluronate and corticosteroid injections, arthrocentesis as well as arthroscopy. However, these treatments are only palliative. Unfortunately, since fibrocartilage lacks an intrinsic ability to self-repair following disease or injury and because the pathogenesis of TMD is multifactorial and incompletely understood, therapy choices for severe cases are few, widely varied and have limited success rates. Severe TMDs often progress until the patient has no choice but to have a discectomy performed, exposing the joint surfaces to direct mechanical loading and abrasion, which will further result in the development of osteoarthritis and may, ultimately, lead to total joint replacement. The use of alloplastic total joint prostheses is reserved as an option of last resort for a small subset of patients, creating a gap in terms of treatment options between non-invasive or minimally invasive strategies and end-stage surgical techniques. The treatments described above do not provide mid-stage intervention for patients. To fill this gap, the implantation of a viable TMJ disc equivalent may improve current treatment modalities by restoring the joint functions provided by a healthy TMJ disc.

Tissue engineering of the TMJ disc aims to regenerate or replace pathological tissues in TMD to restore long-term function. Additionally, research revealed that growth factors and therapeutic drugs added to the engineered disc are released *in situ* to participate in cartilage repair. Even if biomimetic constructs are engineered, a looming challenge is that of surgically accessing the ailing TMJ tissues and integrating the engineering disc within the native milieu. Indeed, it is important to develop novel surgical techniques and associated fixation methods toward human clinical trials.

David Reed: What are some of the design challenges associated with scaling-up these scaffold-based approaches to clinically relevant sizes and how are diffusion limitations associated with size being addressed?

Authors: The scaffold-based tissue engineered TMJ disc strategy can achieve customised size and match clinical needs by selecting appropriate biomaterials and bio-manufacturing technologies. For example, in the study by Legemate *et al.* (2016), the scaffold was prepared by computer design and 3D printing technology, and exogenous growth factors were added to favour the secretion of the matrix from the seeded cells, which not only improved the mechanical properties of the scaffold but also formed the biomimetic joint disc in macro-size.

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