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Review

Exploiting biomaterial approaches to manufacture an artificial trabecular meshwork: A progress report

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ABSTRACT

Glaucoma is the second leading cause of irreversible blindness worldwide. Glaucoma is a progressive optic neuropathy in which permanent loss of peripheral vision results from neurodegeneration in the optic nerve head. The trabecular meshwork is responsible for regulating intraocular pressure, which to date, is the only modifiable risk factor associated with the development of glaucoma. Lowering intraocular pressure reduces glaucoma progression and current surgical approaches for glaucoma attempt to reduce outflow resistance through the trabecular meshwork. Many surgical approaches use minimally invasive glaucoma surgeries (MIGS) to control glaucoma. In this progress report, biomaterials currently employed to treat glaucoma, such as MIGS, and the issues associated with them are described. The report also discusses innovative biofabrication approaches that aim to revolutionise glaucoma treatment through tissue engineering and regenerative medicine (TERM). At present, there are very few applications targeted towards TM engineering *in vivo*, with a great proportion of these biomaterial structures being developed for *in vitro* model use. This is a consequence of the many anatomical and physiological attributes that must be considered when designing a TERM device for microscopic tissues, such as the trabecular meshwork. Ongoing advancements in TERM research from multi-disciplinary teams should lead to the development of a state-of-the-art device to restore trabecular meshwork function and provide a bio-engineering solution to improve patient outcomes.

1. Introduction

Glaucoma is the second leading cause of irreversible blindness worldwide, affecting 64.3 million in 2013 and is estimated to rise to 76 million in 2020 [1]. The use of biomaterials to prevent and restore vision loss within the anterior segment of the eye has been extensively studied in recent years [2]. The anterior segment is comprised of, but not limited to, the cornea, conjunctiva, trabecular meshwork, iris, ciliary body and lens (Fig. 1A) [3]. The trabecular meshwork (TM) is a complex, porous tissue which bridges the iris to the peripheral cornea and plays a vital role in the drainage of aqueous humour into the vascular system (Fig. 1B). The maintenance of a healthy TM is imperative for the homeostasis of intraocular pressure (IOP) with the normal pressure range falling between 10-21 mmHg [4]. Elevated IOP is a major risk factor for glaucoma and is a consequence of TM dysfunction. Elevated IOP results from increased aqueous humour outflow resistance, a result of several morphologic and biochemical changes in the trabecular meshwork (TM); changes in the number of TM cells and the extracellular matrix (ECM) within the TM [5]. There is overwhelming evidence from several prospective randomised multi-centre studies which demonstrate the reduction of IOP is neuro-protective and delays or prevents the structural and functional damage of optic nerve axons in glaucoma [6]. Biomaterial devices have been utilised as an alternative to traditional surgical procedures for long-term maintenance of adequate IOP, but accumulation of scar tissue can also cause these devices to fail [7]. A biomaterial approach that more specifically targets the TM directly is an intervention that may halt the advancement of glaucoma and repair the diseased tissue. Recent research has shown potential for TM tissue repair through stem cell therapy to prevent glaucoma-associated vision loss [8]. There is also evidence of TM progenitor cells which can differentiate into functioning TM cells [9]. Certain biofabrication techniques can generate scaffolds with an environment that closely imitates the extracellular matrix (ECM) of human tissue and incorporates spatial and topographical cues to support stem cell or progenitor cell differentiation into the native phenotype thereby generating a cellular response as observed *in vivo* [10]. By utilising biomaterials and a viable population of TM progenitor cells, a delivery vehicle for stem cell therapy could be generated. However, not all biomaterial techniques are suitable for clinical application, but still could be utilised to develop biomimetic 3D

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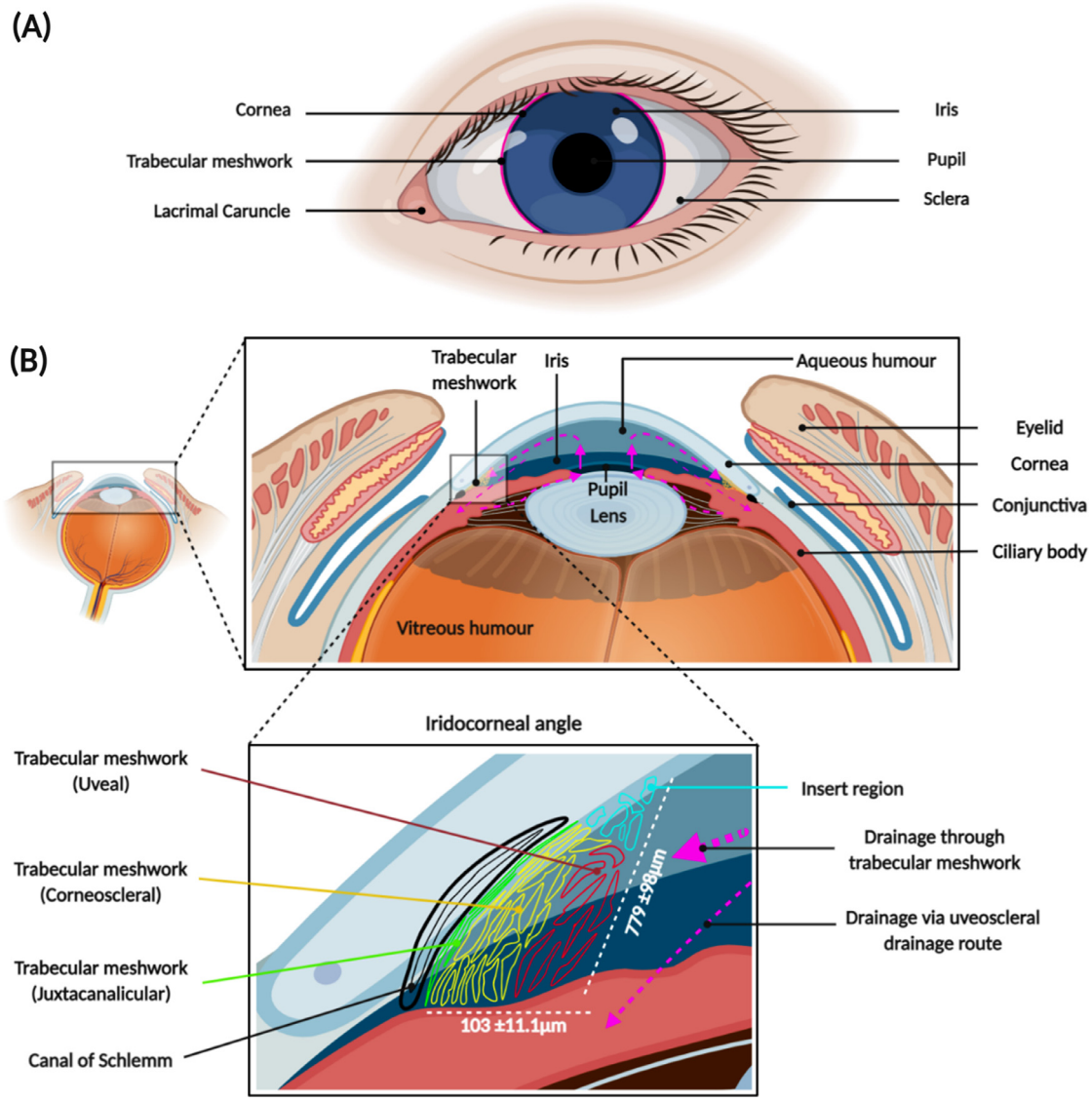

Fig. 1. Schematic diagrams demonstrating (A) the location and circumferential nature of the trabecular meshwork (pink circle). (B) The anterior segment of the eye and its constituents. The magnified view represents the iridocorneal angle highlighting the composition of the anterior chamber and the outflow pathway of aqueous humour (pink arrows indicate flow direction). Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in vitro models to further our understanding of TM tissue biology and the outflow physiology.

This report details the progression of surgical glaucoma treatments and the development of minimally invasive glaucoma surgery (MIGS) devices which exploit biomaterial techniques and discusses their current benefits and aspects that need to be improved. Furthermore, new tissue engineering and regenerative medicine (TERM) approaches that mimic human TM ultrastructure, including essential structural and mechanical properties required, are discussed.

1.1. Trabecular meshwork anatomy

There are many excellent reviews detailing TM biology, [11–13] but a brief overview of TM anatomy pertinent to bioengineering is described herein. The TM is an avascular tissue that spans across the scleral spur to Schwalbe's line with a mean width of 779±98 µm [14] and thickness of 103±11.1 µm [15]. It is composed of three anatomically different "filter" regions made up of connective tissue beams of lamellae and perforated sheets which comprise the uveal meshwork (UM), corneoscleral (CS) and juxtacanalicular (JCT) portions [11]. There is a fourth "non-filter" region located where the TM inserts under the periphery of the corneal endothelium, aptly named the "insert region" and there is evidence this is the location of the progenitor cell niche [12]. Both the UM and CS are composed of multiple layers of connective lamellae that form a highly porous network, allowing free flow of aqueous humour with little resistance. The UM faces the anterior chamber and is the outermost region of the TM, it consists of connective lamellae 25.5±15.6 µm in diameter, which create large intra-trabecular spaces (42.6±19.6 µm). The deeper CS is comprised of flatter lamellae, which results in a more densely packed portion with smaller intra-trabecular spaces (8.9±2.9 µm) [16]. The final filtering portion of the TM is the JCT, which is also the thinnest, spanning 10.1±3.2 µm [17]. The JCT comprises amorphous and irregular cellular sheets held in a loose connective elastin fibre network with beam widths of 4.7±0.8 µm and intra-trabecular spacing ranging between 0.5 and 2 µm [18]. The JCT is positioned adjacent to the inner wall endothelium of Schlemm's canal (SC) and together

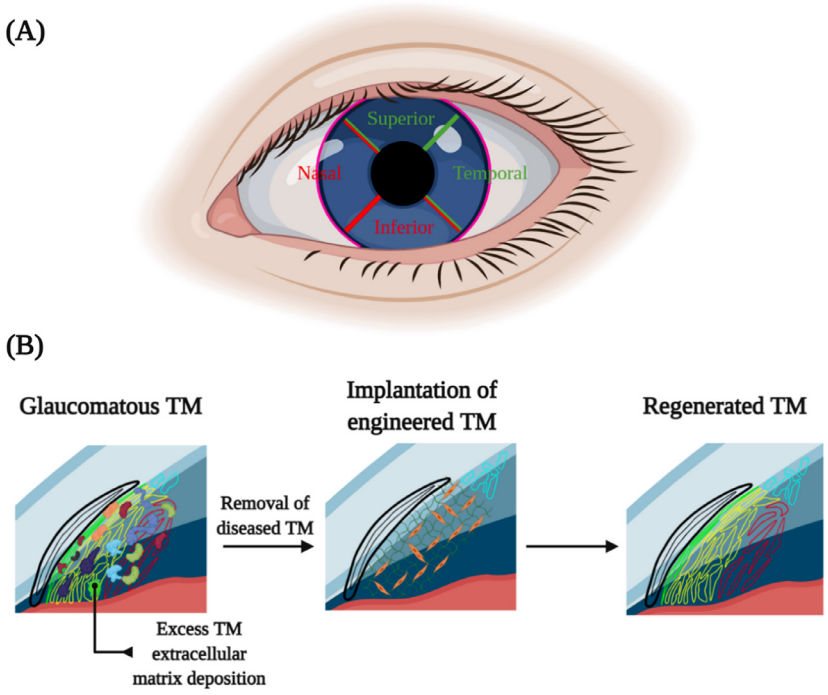

Fig. 2. (A) The eye quadrants demonstrating favourable (green) and unfavourable (red) target sites for glaucoma therapy. [25] (B) Schematic illustrating how an implanted TERM device could regenerate diseased TM tissue. Created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these components comprise the principal site for outflow resistance of aqueous humour.

1.2. Surgical approaches: past, present and future

Anti-glaucoma treatments aim to reduce IOP by 30-50% and slow the progression of disease [19]. The first line treatment is topical pharmacological agents delivered as eye drops to lower IOP, but this often results in poor patient adherence, thus diminishing clinical efficacy and requiring additional surgical intervention to further reduce IOP [20]. Second-line treatments often result in invasive surgical procedures which target IOP reduction in one of two ways; (i) generate a new channel or 'bleb' under the sclera through which fluid can drain more easily, or (ii) insert a tube or shunt into the anterior chamber allowing fluid to bypass the diseased TM tissue. The former, known clinically as "trabeculectomy" is the gold standard procedure for relieving IOP in glaucoma patients [21]. However, this invasive treatment can result in postoperative complications including hypotony, bleb leakage and incidence of fibrosis [21]. Despite the continuous technical refinement of trabeculectomy, the primary goal to achieve target pressure without the need for additional topical medication (complete success) is missed in 35-43% of the patients according to a 20-year follow-up study and postoperative scarring remains the major impediment to higher success rates [22].

Recently, a plethora of MIGS procedures have emerged that all aim to increase aqueous humour outflow through various mechanisms of action [23]. MIGS can be divided into several categories, including micro-trabeculectomy, trabecular bypass operations (stents) and internal or suprachoroidal shunts. The premise of MIGS procedures is to lower IOP through extending the physiological outflow or creating an alternative channel for aqueous humour [24]. The majority of MIGS procedures favour the superior approach, both for practicality, as the nose hinders the nasal and inferior quadrants, and to target more collector channels, thus increasing the efficacy (Fig. 2A) [25]. Currently, there are five commercially available MIGS that reduce IOP through increasing either trabecular (iStent, iStent inject and Hydrus microstent) or subconjunctival (Xen Gel Stent and InnFocus) flow. An overview of their structural properties, risks, benefits and potential complications is discussed in Table 1.

1.2.1. Advancing MIGS suitability

MIGS have undoubtedly revolutionised glaucoma therapy over the past decade. However, a common complication with the subconjunctival-based devices is accumulation of fibrotic tissue leading to failure of the inserts, thought to be caused by the stiffness of the materials used to manufacture the devices, as these are often hard metals or plastics [26]. Whereas, MIGS that increase trabecular flow must cannulate SC, but this is not always potent enough to treat glaucoma [25]. Furthermore, long term follow up and well-designed randomised controlled trials are required to establish long term efficacy and safety. This was highlighted by the clinical withdrawal of the Cypass micro-stent in 2018 due to corneal endothelial cell loss.

An ambitious objective for new and innovative glaucoma surgical approaches, called the "10-10-10" scheme, where the procedure would take <10 minutes to perform, reduce IOP to <10 mmHg and be efficient for >10 years without adverse effects has been advocated. [27] The average candidate for MIGS surgery has a preoperative IOP between 20-25 mmHg, requiring at least a 50% reduction to achieve the postoperative 10 mmHg threshold, but current MIGS procedures rarely reduce IOP by this amount [25]. Additionally, MIGS procedures are much shorter than the traditional trabeculectomy, varying between 15-30 minutes in length. Although MIGS are considered safer than traditional procedures, they are still insufficient compared to the required 10-10-10 criteria. Once the 10-10-10 has been achieved postoperative care should subsequently be reduced, therefore relieving some of the demand on surgeons and allowing more patient procedures to be conducted. Thus it is paramount to manufacture a one-off surgical implant to treat glaucoma that meets these ambitious criteria. Moreover, this would be particularly beneficial for developing countries, such as Africa, where the incidence of glaucoma is estimated to be 11.1 million and healthcare provision is limited [28].

2. Biomimetic scaffold design for TM repair

Tissue engineering and regenerative medicine (TERM) devices aim to restore, imitate or improve tissue function through influencing physical, chemical and biological responses from a cell population grown on a material to prompt normal cell behaviour *in vitro* [33]. Furthermore, TERM

Table 1
Overview of current, commercially available minimally invasive glaucoma surgery devices and procedures.

Commercial Name (and manufacturer)	Device description	Device Illustration	Device dimensions	Device overview & surgical procedure	Benefits	Complications	References
iStent (Glaukos, 2012, 1st generation)	L-shaped, single-piece small tube composed of heparin-coated titanium		Length: 1 mm Height: 0.33 mm Lumen: 0.12 mm	Creates a bypass channel between the anterior chamber and Schlemm's canal to improve aqueous humour drainage through insertion into the TM, using a single-use inserter with a rotator grip for easy handling	Number of postoperative medications reduced Comparable reduction of IOP to trabeculectomy procedure 30-minute procedure No bleb formed	Requires surgical experience Bleeding in anterior chamber (hyphaema) Stent malposition Corneal oedema	[29]
iStent inject (Glaukos, 2018, 2nd generation)	Plug-shaped device made of medical grade titanium and heparin-coated		Length: 0.36 mm Width: 0.23 mm Lumen: 0.08 mm Flow outlets (x4): 0.05 mm	Linear dual stent system inserted using single-use injector device 60º to 90º away from each other for increased outflow facility and easier insertion	Smaller learning curve for second generation device due to smaller size Dual insertion increases overall drop of IOP Lower incidence of complications compared to first generation stent	Hyphaema Hypotony Stent malposition Corneal oedema	[29]
Hydrus microstent (Ivantis, 2018)	A crescent shaped microstent composed of nitinol (nickel-titanium alloy) with four evenly distributed windows for greater access to collector channels		Length: 8 mm Inlet width: 0.29 mm	Device is inserted *ab interno* using a pre-loaded stainless-steel cannula through the TM and follows the curve of Schlemm's canal promoting cannulation and increases fluid outflow by gaining access to more collector channels	Covers 8mm of the TM (approximately a quarter of the total length) to grant access to multiple collector channels and increase outflow facility More area covered decreases chance of canal compression	Hyphaema Corneal oedema Decrease in visual acuity Endothelial cell loss	[30]
Xen Gel stent (Allergan, 2016)	A microfistula tube composed of glutaraldehyde cross-linked porcine gelatine		Length: 6 mm Width: 0.15 mm Lumen: 0.045 mm	The tube is implanted using a pre-loaded injector *ab interno* permitting bleb formation in low-lying drainage space without conjunctiva dissection or sclera flap	No conjunctival dissection or sclera flap opening Bleb formation is low-lying and hidden 15-minute procedure time allows higher patient throughput	Hypotony Choroidal detachment Device erosion or exposure Bleb leakage	[31]
InnFocus (Santen, 2012)	A flexible, needle-like microshunt composed of poly(styrene-*block*-isobutylene-*block*-styrene) with a planar fixation "fin" to prevent migration		Length: 8.5 mm Width: 0.35 mm Lumen: 0.07 mm Fin width: 1.1 mm	The Microshunt is implanted *ab externo* into the anterior chamber permitting subconjunctival fluid flow into a filtration bleb without creation of scleral flap	Fin prevents migration of device and fluid leakage Most potent implant of all MIGS procedures 12-minute procedure time allows high patient throughput	Choroidal detachment Hypotony Hyphaema	[32]

devices should be biocompatible, biodegradable, non-toxic, mechanically robust and biomimetic in their design. Multiple features of TM architecture can be replicated using biofabrication techniques, which could support the growth of phenotypically-appropriate TM cells *in vitro* and their subsequent implantation, where these cells may then repopulate and regenerate the diseased tissue and subsequently restore the outflow pathway and reduce IOP (Fig. 2B). Both structural and mechanical aspects need to be considered when designing a sophisticated TERM device for TM, including pore size, porosity, scaffold dimensions (lamellae width and overall thickness) and inherent stiffness, all of which will now be discussed.

The TM possesses a multi-zonal, complex architecture and the size of the pores and lamellae width in the different filter regions decreases from the outermost UM towards the deeper JCT, producing a porosity gradient [13]. This is a consequence of increased packing density of the beams warranted by a decrease in thickness of the differing filter regions, whereby the combination of a thinner JCT region with smaller intra-trabecular spacing or 'outflow channels' leads to a more closely packed fibril network. Replicating the tissue's porous gradient is an essential property for successfully developing a fully functional bioengineered TM. Biofabrication techniques would need to manufacture a multi-layered system of defined region thickness and decreasing beam width to manipulate and stimulate native cellular behaviour as observed *in vivo*. This has been previously demonstrated in tissue engineering of trabecular bone, where replication of pore size and lamellae thickness ensured topographical, physical and spatial cues guided desirable cell phenotype and tissue-specific gene expression leading to organised tissue growth [34,35].

Fabricating a biomimetic scaffold of the TM should ensure the preservation of *in vivo* cellular activity which is essential to the tissue's functionality in maintaining a competent outflow facility. A key function of TM cells is the phagocytosis of cellular debris (e.g. melanin) from the aqueous humour preventing blockages of the outflow channels. Furthermore, TM cells maintain a homeostatic environment by continually secreting and remodelling (by matrix metalloproteinases (MMPs)) their ECM to counteract any shift in IOP. This occurs primarily in the JCT region, where TM cells sense a change in IOP and hence increase MMP secretion leading to ECM degradation and remodelling to facilitate aqueous outflow [36]. Furthermore, the primary function of the TM is regulation of aqueous humour outflow to maintain IOP (0.24μL/min/mmHg [37]). Therefore, novel devices should preserve these essential cellular functions and allow fluid flow through its porosity gradient, whilst also providing resistance in order to fully recreate the TM's biomechanics and subsequent impact on cell response.

It is also established that TM cells directly sense and respond to the stiffness and topography of their primary substrate [38]. Changes in ECM composition and mechanical integrity occur because of ageing and/or glaucoma [11]. Last *et al.* [39] used AFM to measure the local stiffness of the TM localised at the JCT and found the elastic modulus increased significantly in diseased TM tissue being 4.0±2.2 kPa and 80.8±32.5 kPa for healthy and glaucomatous TM respectively [39]. This increase in stiffness in glaucoma is caused by ECM accumulating in the JCT region [5]. TM cell dysfunction and ECM remodelling in the JCT region is altered in glaucoma resulting in a considerably stiffer tissue [36]. In terms of bulk tensile properties, Camras *et al.* [40] determined the elastic modulus of fresh TM (with cells) to be 51.5±13.6 MPa. Furthermore, aqueous humour flow is non-uniform around the TM's circumference. There is evidence that suggests these segmental low and high flow regions of fluid influence the biomechanical properties of the TM, whereby the stiffness of the tissue becomes non-uniform throughout. It was found that regions of low flow were up to 2.3-fold stiffer than high flow regions when measured using atomic force microscopy [41]. Therefore, the bulk and local stiffness of a TERM device needs to suitably match those of healthy TM to trigger appropriate cell behaviour.

Injectable hydrogels, electrospinning, photolithography and freeze casting are the few biofabrication techniques that have been explored for TM engineering to date (Fig. 3). Several of these were discussed by Dautriche *et al.* [42] in 2014 and this report focuses on their current progress, including the applicability of new techniques for developing future TM TERM devices.

2.1. TM biomaterial scaffolds

The first documented approach for TM repair and regeneration utilised a novel, biomimetic peptide hydrogel [43]. Hydrogels are three-dimensional porous networks composed of hydrophilic polymer cross-links that swell in water whilst maintaining their original structure. [44] They have received considerable attention in TERM due to their ability to mimic natural ECM, provide structural integrity, promote cellular organisation and morphogenic guidance and to encapsulate and deliver cells without initiating an immunological response [45]. As such, hydrogels have multiple biomedical applications, including drug delivery, wound healing and as ophthalmic materials [46]. Schlunck *et al* reported stiffer hydrogels cause TM cell-matrix interactions, cytoskeletal structures, signal transduction and protein expression patterns similar to those observed in glaucoma when compared to softer gels [47]. This further demonstrates the direct influence hydrogels can have on TM cell activity.

Waduthanthri *et al* [43] bioengineered a 3D TM scaffold using a modified shear-thinning peptide hydrogel system, MAX8B (a peptide blend of MAX8 and MAX8-GRGD (9:1)) to be utilised as an *in vitro* model but could also function as an injectable implant. The MAX8 peptide is comprised of 20 alternating hydrophobic and hydrophilic amino acids (lysine and valine) that self-assemble to create a nanofibrillar network that resembles TM ECM. The MAX8-GRGD is a peptide extension of the original MAX8 compound, where the GRGD sequence facilitates human TM cell interactions through focal adhesions and improves overall biocompatibility. Human TM cells cultured *in vitro* for 7-days within the hydrogel actively secreted collagen IV and fibronectin throughout its 3D structure and yielded a TM-like stiffness of 1.37±0.02 kPa, which was also four times greater than the hydrogel free from cells. Furthermore, a suitable injectable hydrogel must possess the ability to become a fluid during injection (shear-thinning) and demonstrate fast recovery to its original nanostructure once delivered [48]. The cell-seeded MAX8B system exhibited shear-thinning properties appropriate for clinical use when passed through a 31-gauge needle into a vertically orientated tissue culture plate [49].

Whilst an injectable hydrogel is a promising start for generating an appropriate *in vivo* therapeutic, this system does not come without its own limitations, such as poor mechanical properties, which often limits these materials to soft and non-load bearing tissues [50]. This could hinder the ability of the hydrogel to be a successful implantation device in future studies due to the contractile nature of the TM and constant flow the biomaterial will be subjected to. Therefore, this system may be best employed in combination with trabeculectomy to deliver healthy cells directly to the incision site to aid postoperative recovery and eliminate the need for follow-up surgeries. Further studies, such as successful delivery of hydrogel and prolonged cell viability in a suitable animal model, will need to be conducted before the MAX8B system can be deemed as a suitable therapeutic for glaucoma treatment.

Electrospinning is a promising and versatile biofabrication technique which has been employed for TM repair after receiving notable attention in TERM applications, including bone, skin and cardiovascular tissues [51]. Electrospinning produces micro/nano-fibrous scaffolds from either natural or synthetic polymer solutions by utilising electrostatic forces [52]. Originally developed for filtration, electrospinning has become increasingly useful in TERM research, owing to its high surface area to volume ratio supporting cell attachment and mimicking the host tissues ECM [53]. Electrospun scaffolds are also mechanically strong and confer contact guidance and directionality to seeded cells, making it an attractive technique to fabricate an artificial TM [54]. However, these porous scaffolds allow minimal to no cellular integration as their densely

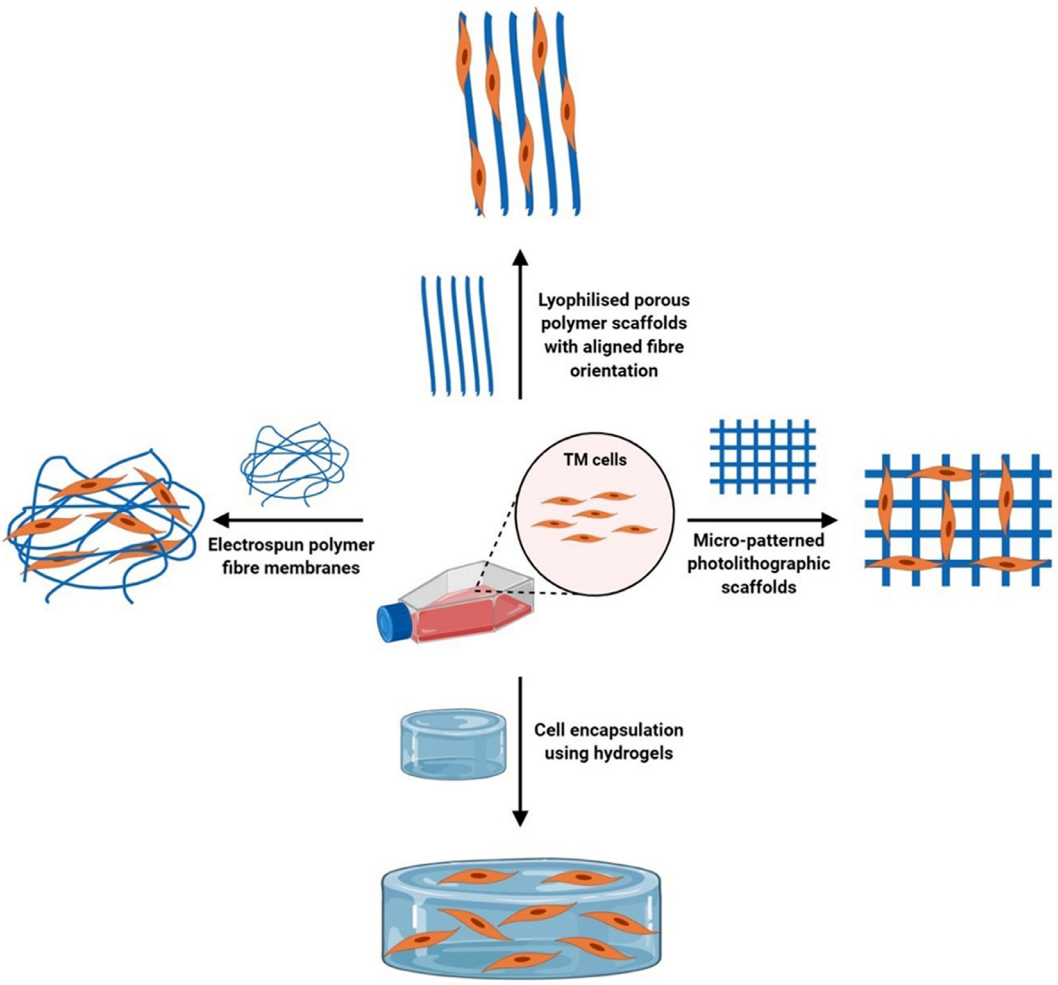

Fig. 3. Biofabrication techniques currently exploited for both *in vitro* and *in vivo* TM engineering. Created with BioRender.com.

packed fibre networks often results in small pore sizes that impedes cell infiltration [55].

Klapstova *et al* [56] have recently developed an alternative to MIGS devices using electrospinning. Their scaffold aimed to be biocompatible, non-degradable and cell growth resistant, which has demonstrated the ability to prevent a fibrotic reaction that would lead to the blockage of the implant [57]. They electrospun non-degradable polyvinylidene-fluoride (PVDF) with polyethylene oxide (PEO) to fabricate a 3D fibril network, with average fibre diameter of 1.12±0.004 μm. Mouse 3T3 fibroblasts cultured on this scaffold for 8 days demonstrated minimal cell proliferation, which was attributed to partially washed out PEO changing the surface properties of the fibres and therefore suppressing cell growth. Although this electrospun scaffold has demonstrated favourable resistance to cellular proliferation, further studies still need to be completed, most notably human TM cell culture, as this study only explored mouse 3T3 fibroblasts, and culture over longer time periods to ensure continued growth restriction. Interestingly, electrospun scaffolds have been previously reported to facilitate TM cell adhesion and expansion, demonstrating the suitability of these polymeric biomaterials to TM bioengineering [58].

Despite rapid progress in biofabrication technologies, the number of studies aiming to develop therapeutic systems for TM are still vastly lacking. Instead, application of other biofabrication techniques have been focused on generating sophisticated 3D models to better understand TM biology *in vitro*. The use of these techniques and the possibility of adapting them to create viable TERM devices for application *in vivo* will be discussed.

Photolithography utilises selective exposure of light sensitive polymers to transfer user-generated geometric patterns onto a substrate to create three-dimensional micro- or nano-environments [59]. Photolithography involves three basic process steps (coat, develop and expose) to transfer a pattern from a mask to a photosensitive layer leading to fabrication of a 3D multi-layered hydrophobic scaffold. As such, photolithography is a powerful technique for fabricating biomimetic scaffolds, as it permits complete control of essential structural aspects, such as pore size, pore shape, porosity, fibre diameter and overall thickness [60]. These qualities suggest that photolithography would be a viable technique to yield an artificial TM.

In 2013, Torrejon *et al* created photolithographic biocompatible scaffolds from epoxy, negative photoresist SU-8 polymer with a repeat square pore size of 12 μm, beam width of 7.3±0.1 μm and overall thickness of 20 μm to be employed as a well-defined 3D *in vitro* model [61]. The dimensions of this porous biomaterial resembled native TM and cultured human TM cells exhibited physiological activity such as building resistance to fluid flow. Whilst the pore size and fibre diameter are likened to the native TM, the overall scaffold thickness is insufficient in fabricating an appropriate biomimetic material. Therefore, in their current form, these materials would not be suitable for *in vivo* implantation as the TM is approximately five times thicker. Furthermore, in a separate study where SU-8 was used, scaffolds with a similar thickness of 25 μm were achieved [62]. However, these SU-8 scaffolds yielded an elastic modulus of 2.2±0.1 GPa through tensile testing, which is considerably stiffer than that of the human TM. This imbalance could have a

significant impact on TM cell response due to the known effect stiffness can have on cell activity [5].

Freeze-casting is another biofabrication approach that has been recently employed for TM engineering [63]. Freeze-casting is a relatively simple process that generates a unique porous biomaterial by flash-freezing a polymer suspension, followed by sublimation and sintering, where the porosity of the scaffold directly replicates the frozen solvent crystals [64]. This technique can produce well-controlled porous structures which replicate the intricate ECM of complex tissues. Furthermore, scaffolds generated by this approach are deemed to have excellent biocompatibility and biodegradability properties [65].

In 2017, Osmond *et al* [63] used freeze-casting to generate a collagen-based, uniaxially aligned, porous biomaterial with chondroitin sulphate (ChS; a glycosaminoglycan) incorporated for TM engineering. Collagen and ChS are both ECM components of the TM and, therefore make a viable combination to employ for its bioengineering [11]. The study generated both collagen-only and collagen-ChS scaffolds that were comparable to the human TM tissue, with pore size 10.25±5.1 μm and 9.48±4.7 μm and elastic moduli (compressive dynamic mechanical analysis) 6.71±3.2 kPa and 6.73±1.7 kPa, respectively. These physiologically-relevant biomaterials permitted migration of porcine TM cells over a 2-week period and cells were observed growing along the aligned fibrillar network, like their growth in native tissue. Interestingly, the same group later reported that structural features, such as pore size and alignment of fibres, were just as influential in guiding TM cellular activity as the incorporation of glycosaminoglycans [66]. Further long-term studies, including the flow of fluid through its porous structure, are required in order to better determine the applicability of this technique and these scaffolds.

All of these biofabrication methodologies have their own unique advantages for TM engineering. Collectively, these techniques all support TM cell adhesion and expansion, as well as promote cellular activity as observed *in vivo*. However, aside from the injectable hydrogel system, the ability to advance these structures for *in vivo* application requires their method of delivery to the site of implantation to be given due consideration and should be developed with ophthalmic specialist input from the outset to ensure clinical translation and device efficacy.

3. Conclusion

This report has outlined the complexity of the TM's architecture, the challenges that are faced with current therapies and how exploiting biomaterial development could revolutionise the way in which glaucoma is treated. We have also discussed the key attributes that a TERM device would need to imitate in order to manufacture a successful and innovative therapeutic for glaucoma treatment. Current biofabrication techniques employed for TM engineering have taken a step in the right direction and display great promise in generating a device that could support and aid TM cell growth and activity. The prevalence of glaucoma continues to advance, yet the potential of these biomaterial systems - if incorporated into current surgical procedures - could have a significant impact in the treatment of glaucoma, including one-off surgeries and reduced need for postoperative interventions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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