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ECM biomaterials for modeling of outflow cell biology in health and disease

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ABSTRACT

This review highlights the importance of extracellular matrix (ECM) biomaterials in understanding the biology of human trabecular meshwork (TM) and Schlemm's canal (SC) cells under normal and simulated glaucoma-like conditions. We provide an overview of recent progress in the development and application of state-of-the-art 3D ECM biomaterials including cell-derived ECM, ECM scaffolds, Matrigel, and ECM hydrogels for studies of TM and SC cell (patho)biology. Such bioengineered platforms enable accurate and reliable modeling of tissue-like cell-cell and cell-ECM interactions. They bridge the gap between conventional 2D approaches and *in vivo/ex vivo* models, and have the potential to aid in the identification of the causal mechanism(s) for outflow dysfunction in ocular hypertensive glaucoma. We discuss each model's benefits and limitations, and close with an outlook on future directions.

Search strategy

We searched PubMed for the following keywords, in different combinations: "human trabecular meshwork cells, human Schlemm's canal cells, 3D *in vitro* model, bioengineering, biomaterials, cell-derived matrix, ECM scaffolds, ECM hydrogels, Matrigel" as of December 2023. Herein, we summarize 17 primary research reports, excluding news stories or meeting abstracts.

Data availability

No data was used for the research described in the article.

1. Introduction

Situated at the iridocorneal angle in the anterior chamber of the eye (Fig. 1), the trabecular meshwork (TM) and Schlemm's canal (SC) inner wall endothelium form the central functional unit of the conventional outflow pathway [1,2]. The two tissues jointly regulate aqueous humor outflow resistance and thereby intraocular pressure [3], with the bulk of this resistance localized to the deepest aspect of the juxtacanalicular tissue (JCT) region of the TM and SC inner wall basal lamina [4,5] (Fig. 2). The complex structural and biochemical environment of the multi-layered TM and SC is governed by a specialized extracellular matrix (ECM). The porous inner uveal trabecular meshwork and central corneoscleral trabecular meshwork consist of TM cell-lined trabecular beams composed of collagen types I and III and elastin fibers. Collagen type IV and laminin are found in the TM cell basal lamina [2,6,7]. The outer JCT does not form trabecular beams. Instead, it consists of few discontinuous TM cell layers embedded in an amorphous dense ECM made of, for example, fibrillar and non-fibrillar collagens, elastic fibrils, and fibronectin. The narrow space between TM cells and ECM fibers contains a ground substance rich in glycosaminoglycans, proteoglycans, and matricellular proteins [8].

Specifically, collagens I, III, and IV play important roles in structural integrity of the ECM throughout the TM and the SC inner wall basal lamina, elastin aids in TM flexibility, and fibronectin facilitates cell adhesion. Highly charged glycosaminoglycans such as hyaluronic acid, chondroitin sulfate, and dermatan sulfate influence TM-ECM viscoelastic properties, with heparan sulfate modulating SC cell connectivity. Proteoglycans such as biglycan, decorin, and versican regulate collagen

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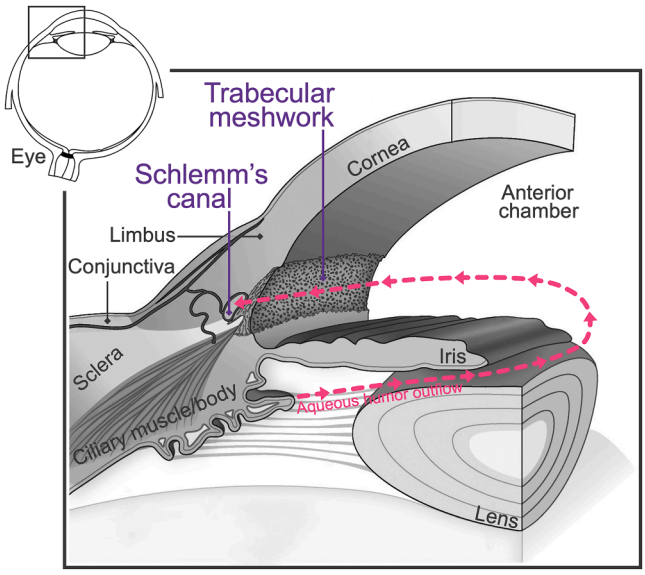

Fig. 1. Anterior segment architecture. The anterior segment of the human eye is composed of the cornea, anterior chamber, iris, lens, ciliary muscle/body, and conjunctiva. The limbus represents the transition between peripheral cornea and sclera that contains the aqueous humor outflow structures, including the TM and SC (reproduced and adapted from [9] with permission from Proceedings of the National Academy of Sciences under Creative Commons license).

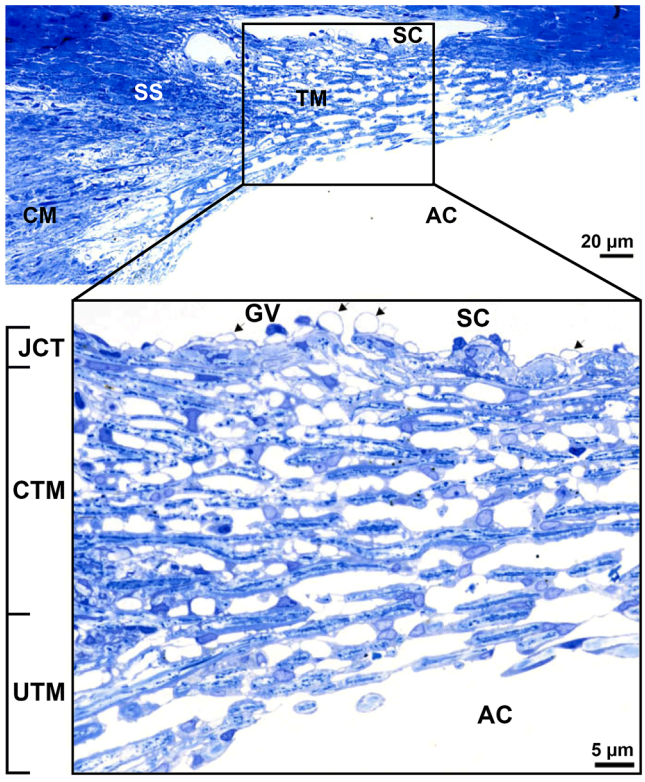

Fig. 2. Outflow tissue architecture. Light micrograph of a meridional section through the TM and SC (exhibiting two lumens in the plane of this section, separated by a septum). SS: scleral spur; CM: ciliary muscle; AC: anterior chamber; GV: giant vacuole (arrows); JCT: juxtacanalicular tissue; CTM: corneoscleral trabecular meshwork; UTM: uveal trabecular meshwork (reproduced and adapted from [2] with permission from Elsevier).

spacing and thereby impact ECM structure [5,6,8,10–12]. Collectively, these structures govern the delicate balance of aqueous humor outflow through dynamic interactions between TM and SC cells with their ECM microenvironment. Taking into account the composition and architecture of the ECM and its complex regulatory roles is therefore vital for studies to improve our understanding of outflow tissue physiology and pathophysiology.

This review focuses on ECM biomaterials and their utility in studies of TM and SC cell biology under normal and simulated glaucoma-like conditions. Pathological alterations in the outflow pathway are responsible for increased outflow resistance in ocular hypertensive glaucoma, the leading cause of irreversible blindness affecting approximately 80 million people worldwide [3,13]. However, there is a fundamental lack of understanding how TM/SC cells and their ECM milieu develop glaucomatous dysfunction. While important from a physiological standpoint, *in vivo* animal and *ex vivo* organ culture models do not facilitate detailed investigations of dynamic cell-ECM interactions in the outflow tract. Similarly, 2D *in vitro* TM and SC cell monolayer cultures using conventional plastic or glass substrates have proven value; however, they cannot replicate the outflow tissue's ECM makeup and dimensionality. Therefore, we highlight the necessity of creating 3D models based on biomaterials to overcome these limitations. Such bioengineered systems facilitate accurate and reliable modeling of tissue-like cell-cell and cell-ECM interactions bridging the gap between conventional 2D approaches and *in vivo/ex vivo* models.

In contrast to existing literature, the purpose of this review is to specifically emphasize the novel contributions of 3D ECM-based models in elucidating the mechanisms underlying human TM and SC cell biology in health and disease. For other biomaterials-based models, we refer to a recent publication [14].

2. ECM biomaterials to study TM cell biology

Most *in vitro* studies to date have focused on the TM, owing to both its central role in outflow regulation and broad accessibility of donor-derived primary cells. In the following section, we discuss the application of 3D ECM-based models to interrogate human TM cell (patho) biology.

2.1. Cell-derived ECM

TM cell-derived ECM comprises a complex array of higher-order ECM molecules deposited by TM cells over time *in vitro* [15]. Upon decellularization, these ECM structures present a relevant microenvironment to re-seeded TM cells that is rich in topography, chemistry, and whose mechanics can be tuned to approximate the network of native ECM constituents.

Raghunathan et al. [16] used glaucomatous TM cell-derived ECM to determine the effects of a tissue-like pathomimetic matrix on normal TM cell behavior. The study found that ECM deposited by glaucomatous TM cells was stiffer – in turn inducing stiffening of re-seeded TM cells – and resistant to cellular remodeling compared to normal TM cell-derived ECM. This was accompanied by expression differences in matrix and matricellular proteins, and the contractile cytoskeletal protein alpha smooth muscle actin (αSMA) implicated in fibrotic diseases. Furthermore, glaucomatous TM cell-derived ECM induced endoplasmic reticulum stress in normal TM cells, as demonstrated by increased expression of key markers involved in the "unfolded protein response" mechanism. These phenomena were generally exacerbated by co-treatment with a glucocorticoid. The authors conclude that TM cell-derived ECM materials accurately mimic the glaucomatous extracellular milieu to facilitate detailed studies of the bidirectional interactions between TM cells and their immediate microenvironment.

In another study, Yemanyi et al. [17] investigated whether genipin-crosslinked TM cell-derived ECM initiates a fibrotic-like phenotype in re-seeded TM cells via dysregulated mechanosignaling

pathways (Wnt/β-catenin and YAP/TAZ). They found that enzymatically crosslinked TM cell-derived ECM increased the presence of structural and basal lamina ECM proteins in a concentration-dependent manner compared to uncrosslinked controls. This was correlated with increased matrix stiffness, which in turn induced stiffening of re-seeded TM cells. The study also showed impaired activity of β-catenin and YAP/TAZ mechanosignaling in TM cells in response to crosslinked TM cell-derived ECM, concurrent with inactive RhoA/Rho kinase (ROCK) signaling – a key modulator of TM cell cytoskeletal dynamics in response to mechanical signals. Pharmacological activation of the Wnt/β-catenin pathway rescued the stiff-induced aberrant TM cell phenotype. The authors conclude that Wnt pathway activation has the potential to mitigate glaucomatous TM dysfunction induced by crosslinked/stiffened ECM.

Yemanyi et al. [18] then examined the effects of glucocorticoid-induced TM cell-derived ECM on re-seeded normal TM cells in the context of glaucoma-associated transforming growth factor beta2 (TGFβ2). They found that, in the absence of TGFβ2, glucocorticoid-induced TM cell-derived ECM activated non-Smad signaling in TM cells. This was accompanied by upregulation of different structural ECM, ECM remodeling, matricellular, and protein crosslinking genes/proteins. The presence of exogenous TGFβ2 potentiated these fibrotic-like effects via activation of canonical Smad and non-Smad signaling. The study also showed that inhibition of type I TGFβ2 receptor kinase attenuated the induction of key ECM and contractile cytoskeleton components driven by glucocorticoid-induced TM cell-derived ECM. The authors conclude that glucocorticoid-induced TM cell-derived ECM is sufficient to drive aberrant cellular behavior, and that inhibiting TGFβ2 receptor kinase may be a viable therapeutic strategy to treat ocular hypertensive glaucoma.

Lastly, Yemanyi et al. [19] used the same glucocorticoid-induced TM cell-derived ECM to assess its effects on TM cell mechanoreceptors, integrin adhesomes, and actin-related proteins. The study showed that ECM produced by glucocorticoid-induced TM cells increased the deposition of fibronectin in re-seeded normal TM cells compared to controls. This was associated with increased expression of αV integrin, cavin1, and RhoA – all involved in focal contact formation – in a time-dependent manner, whereas αSMA was elevated in a time-independent fashion. They further showed that TM cells respond in a distinctly different manner to glucocorticoid-induced TM cell-derived ECM compared to exogenously supplied, soluble glucocorticoid. The authors conclude that understanding these nuanced cellular responses may help to further elucidate the complex landscape of TM cell-ECM connectivity, potentially providing new avenues for therapeutic strategies.

Taken together, cell-derived ECM from glaucomatous or glucocorticoid-induced TM cells provide a valuable tool for mechanistic investigations of the reciprocal interactions between TM cells and their aberrant microenvironment in glaucoma. Additional disease-associated biochemical (e.g., TGFβ2) or biomechanical (i.e., ECM crosslinking/stiffening) stressors offer further opportunities for nuanced TM cell mechanobiology studies. Limitations to the use of cell-derived ECM include the potential "batch-to-batch" variability contingent on the TM cell strain used (i.e., donor sex/age), and the higher number of cells required first for decellularization then for recellularization compared to other models, posing practical challenges that could hamper reproducibility. Differences in cell culture conditions and decellularization techniques could further introduce undesired inconsistencies across studies.

2.2. ECM scaffolds

ECM scaffolds provide precise control over composition and structure, thereby enabling uniform and reproducible platforms with little "batch-to-batch" variability [20]. Collagen type I is the most abundant fibrillar protein in the human body and main structural element of the outflow tissue ECM. As such, it is strategically incorporated in bioengineered ECM scaffolds.

Osmond et al. [21] fabricated collagen type I scaffolds containing glycosaminoglycans found in the native tissue and different pore architectures using freeze-casting to investigate how seeded TM cells respond to changes in their ECM microenvironment. They found that TM cells grew better on large pore-ECM scaffolds compared to small-pore counterparts, with random pore arrangement having a positive effect on cell proliferation relative to aligned pores. Of the two glycosaminoglycans tested, chondroitin sulfate promoted cell growth whereas hyaluronic acid, or the combination of both, produced the opposite effect. The study also showed that fibronectin expression was increased with glycosaminoglycan incorporation, with its morphology being governed by the underlying pore architecture. The authors conclude that these findings provide new insights into the mechanisms underpinning ECM deposition and turnover in the TM, which may help improve our understanding of cell-ECM interactions in outflow regulation.

Using the same collagen-glycosaminoglycan scaffolds, Adhikari et al. [22] next examined ECM mRNA and protein expression in seeded TM cells and perfusion pressure gradients across the 3D scaffolds, in presence or absence of glaucoma-associated glucocorticoid. The study showed that chondroitin sulfate and hyaluronic acid induced the expression of elastin, laminin, and MMP-2 mRNA – all involved in basal lamina/ECM remodeling; this also affected the morphological distribution of these proteins including aberrant intracellular accumulation compared to collagen-only scaffolds. Glucocorticoid treatment increased the expression of elastin but decreased laminin and MMP-2 expression, with concurrent increase in pressure gradients consistent with glaucomatous tissue behavior. The authors conclude that these data may help to further our understanding of how glycosaminoglycans influence TM cell function in the context of aqueous humor outflow resistance alterations in glaucoma.

Collectively, ECM scaffolds made from collagen and glycosaminoglycans found in the native tissue, in conjunction with soluble glaucoma-associated biochemical stressors (e.g., glucocorticoids), provide a valuable tool to interrogate TM cell-ECM interactions in glaucoma. Incorporation of ECM scaffolds into perfusion bioreactors adds further value for studies under dynamic culture conditions. Limitations to the use of ECM scaffolds include the reductionist nature of the scaffold design that cannot fully recapitulate the complex nature of the native ECM microenvironment, variability in the cell source (so far, only commercial TM cells have been used), and discrepancies in culture conditions that may contribute to potential inconsistencies.

2.3. Matrigel

Matrigel is the gelatinous extract of basement membrane ECM proteins, such as collagen IV, heparin sulfate and nidogen, and various growth factors derived from the Engelbreth-Holm-Swarm mouse sarcoma [23]. The solution forms a solid gel when warmed to 37°C, enabling relatively easy 3D cell cultures in a complex tissue-like environment.

Bouchemi et al. [24] used Matrigel to determine the effects of benzalkonium chloride, a cationic surfactant frequently used as preservative in topical glaucoma medications, on TM cell cytoskeleton remodeling and expression of inflammatory markers in comparison to glaucoma-associated glucocorticoid or TGFβ2 exposure. They showed that treatment with benzalkonium chloride induced TM cell cytotoxicity inside the Matrigel network compared to controls, with remaining cells exhibiting an abnormal cytoskeletal phenotype. Unlike in glucocorticoid- or TGFβ2-treated samples, no crosslinked actin networks were observed with benzalkonium chloride. However, TM cells showed increased mRNA expression of the cytokines interleukin-6 and -8 in a time-dependent manner compared to controls, with no major changes found in matrix metalloproteinase (MMP)-9 levels. This suggests that benzalkonium chloride exposure may elicit an inflammatory response in TM cells without impacting enzyme-mediated ECM degradation

processes. The authors conclude that these findings provide new insights into how preservatives may inadvertently contribute to TM degeneration and increased outflow resistance, possibly reducing the efficacy of common glaucoma medications.

In another study, Vernazza et al. [25] investigated the effects of chronic oxidative stress, frequently associated with glaucoma pathogenesis, on TM cells cultured atop or within Matrigel constructs. The study showed that 3D-encapsulated TM cells produced more reactive oxygen species upon hydrogen peroxide treatment compared to cells cultured as 2D monolayers. This was accompanied by increased metabolic activity in 3D vs. 2D TM cell cultures in response to chronic oxidative stress and further involved the induction of pro-inflammatory interleukins and a distinct anti-apoptotic signature. The authors conclude that these data may help to further our understanding of the impact of chronic oxidative stress on glaucomatous TM dysfunction involving a detrimental inflammatory component.

Using the same 3D Matrigel system, Sacca et al. [26] next examined the effects of long-term oxidative stress on TM cell behavior in both static and dynamic culture conditions using a perfusion bioreactor. They found that chronic oxidative stress caused TM cell F-actin cytoskeleton reorganization with time-dependent partial recovery in perfusion culture. Similar observations were made in terms of metabolic activity. Dynamic culture increased mRNA expression of various pro-inflammatory cytokines and MMPs compared both to untreated controls and static cultures subjected to chronic oxidative stress. This was correlated with increased expression of pro-apoptotic proteins early on, with TM cells gradually counterbalancing this response, as evidenced by increased expression of pro-survival markers. This suggests that glaucoma-associated oxidative stress may elicit only transient and reversible TM alterations. The authors conclude that the Matrigel-based 3D culture platform paired with dynamic perfusion has the potential to further our understanding of key events involved in glaucoma onset and progression.

Lastly, Baffault et al. [27] investigated the effects of clinically-used ROCK inhibition on TGFβ2-induced TM cell dysfunction using 3D Matrigel in comparison to the prostaglandin analog latanoprost. As expected, exposure to TGFβ2 induced TM cell F-actin stress fiber formation, including crosslinked actin networks, and increased the expression of αSMA and fibronectin. This was accompanied by increased myosin light chain and cofilin phosphorylation – two key components of the TM cell actomyosin contractile machinery – and contraction of the 3D constructs. ROCK inhibition reversed the TGFβ2-induced pathological changes to baseline levels, whereas latanoprost only decreased fibronectin deposition but did not affect cytoskeletal remodeling. These data confirm the differential mode of action of the compounds tested, TM-targeting (i.e., ROCK inhibitor) versus TM-non-targeting (i.e., prostaglandin analog). The authors conclude that the Matrigel 3D culture platform has the potential to aid in glaucoma drug development and screening.

Taken together, Matrigel provides a valuable tool for mechanistic studies of the bidirectional interactions of TM cells with their altered ECM milieu in glaucoma when used in combination with soluble glaucoma-associated biochemical stressors (e.g., TGFβ2) and/or perfusion culture. Limitations to the use of Matrigel include the known "batch-to-batch" variability and the inability to control architecture, as well as its tumorigenic origin and undefined composition affecting the biochemical and mechanical properties. This lack of fine control is exacerbated by possible variability in the cell source (commercial versus freshly-isolated TM cells) and culture conditions that may introduce additional inconsistencies.

2.4. ECM hydrogels

ECM hydrogels are water-swollen 3D networks of crosslinked biopolymer chains that provide a simplistic yet tissue-like microenvironment. Physically crosslinked hydrogels are created by reversible intermolecular interactions, whereas chemically crosslinked hydrogels are formed by permanent covalent bonding [28]. Free radical-mediated photopolymerization is a widely-used chemical crosslinking approach to generate ECM hydrogels. Polymer precursors containing photoactive functional groups (i.e., thiols, methacrylates) are chemically crosslinked by short exposure to ultraviolet (UV) or blue light in the presence of a cytocompatible photoinitiator. Therefore, ECM hydrogels support the culture of cells both within the 3D polymer network (i.e., encapsulated before crosslinking) and atop pre-formed constructs (i.e., seeded after crosslinking).

Li et al. [29] first reported an ECM hydrogel composed of TM cells encapsulated in a network of ECM biopolymers found in the native JCT TM region: collagen type I, hyaluronic acid, and elastin (in form of an elastin-like polypeptide) – all containing photoactive functional groups for short UV light-mediated chemical crosslinking. The study showed that TM cells inside ECM hydrogels responded to glucocorticoid treatment acquiring a glaucoma-like phenotype, as evidenced by increased contractility and cytoskeletal/ECM remodeling. These alterations contributed to pathological TM hydrogel condensation and stiffening, consistent with glaucomatous tissue behavior, which was prevented/reversed with ROCK inhibition. Encapsulation of patient-derived glaucomatous TM cells was shown to mirror the glucocorticoid-induced changes while remaining responsive to ROCK inhibition, confirming the bidirectional utility of the hydrogel system to model glaucomatous outflow dysfunction. The authors conclude that the TM cell-encapsulated ECM hydrogel provides a new tool for advanced disease modeling and glaucoma drug screening.

In a similar study, Adhikari et al. [30] used photo-functionalized gelatin (i.e., hydrolyzed collagen) interspersed with unmodified chondroitin sulfate, hyaluronic acid, or the combination of both to fabricate ECM hydrogels via blue light-mediated chemical crosslinking. They found that TM cells seeded atop the pre-formed hydrogels were responsive to the presence of glycosaminoglycans in terms of cell proliferation and fibronectin expression. Upon exposure to glaucoma-associated glucocorticoid, TM cells increased fibronectin mRNA and protein levels (independent of the presence or absence of glycosaminoglycans), consistent with pathological tissue behavior. These data provide new insights into glaucomatous TM alterations at the cellular and molecular level through the lens of glycosaminoglycans. The authors conclude that this ECM hydrogel has the potential to aid in glaucoma drug screening studies.

Li et al. [31] next used their collagen type I, hyaluronic acid, and elastin-like polypeptide hydrogel to investigate the effects of disease-associated ECM stiffening and TGFβ2 exposure on regulating TM cell YAP/TAZ mechanosignaling, and whether YAP/TAZ inhibition could prevent TM cells from acquiring a glaucoma-like phenotype. The study showed that increased ECM rigidity increased TM cell YAP/TAZ nuclear translocation, the principal mechanism regulating their transcriptional activity. This was accompanied by focal adhesion and cytoskeletal reorganization. TGFβ2 treatment increased nuclear YAP/TAZ in both normal and glaucomatous TM cells, which was prevented by blocking ERK and ROCK signaling pathways contingent on F-actin cytoskeleton integrity. When YAP/TAZ were inhibited with siRNA or the clinically-used small molecule verteporfin (without light stimulation), the induced pathological changes were largely reversed to control levels. Similarly, YAP/TAZ inhibition with verteporfin blocked ECM hydrogel contraction and stiffening upon cell encapsulation induced by TGFβ2, supporting the notion that abnormal YAP/TAZ mechanosignaling may play a key role in glaucomatous TM cell dysfunction. The authors conclude that these data may aid in the development of new multifactorial therapeutic strategies to prevent or treat progressive ocular hypertension in glaucoma.

Using the same ECM hydrogel, Li et al. [32] then examined the effects of glaucoma-associated TGFβ2 on TM cell contractility in comparison to conventional glass substrates. They found that cellular and nuclear morphology of TM cells seeded atop pre-formed hydrogels were

affected by the culture substrate, with concurrent alterations in the organization of F-actin. Treatment with TGFβ2 increased TM cell contractility through both ERK and ROCK signaling pathways, as evidenced by targeted pathway inhibition. This was shown to critically involve the contractile actomyosin machinery contingent on the culture substrate. ERK inhibition enhanced TGFβ2-induced phospho-myosin light chain – a key downstream effector of active ROCK – in TM cells grown on ECM hydrogels, but not on glass. This was accompanied by hypercontractility of TM cell-encapsulated hydrogels. ROCK inhibition produced the exact opposite effect and relaxed the contracted TGFβ2-induced ECM hydrogels. These findings provide new insights into how ERK signaling may crosstalk with and thus regulate ROCK-dependent TM contractility. The authors conclude that careful considerations of the culture substrate are warranted for mechanistic interrogations of TM cell (patho)biology.

Lastly, Yoo et al. [33] assessed the connection between YAP/TAZ mechanosignaling and the mevalonate pathway by assessing the effects of clinically-used simvastatin on attenuating glucocorticoid-induced TM cell dysfunction. Statins inhibit HMG-CoA reductase, which catalyzes the production of mevalonic acid in the mevalonate (cholesterol biosynthesis) pathway. The study showed that simvastatin potently blocked aberrant YAP/TAZ nuclear localization/activity and actomyosin contractility. Simvastatin co-treatment mitigated the glucocorticoid-induced ECM hydrogel contraction and stiffening upon cell encapsulation, concurrent with reduced ECM deposition. Sequential simvastatin treatment (i.e., following glucocorticoid induction) was similarly effective but did not match ROCK inhibition. The authors conclude that these data may help to further our understanding of the association of statin use with a reduced risk of developing glaucoma via modulating YAP/TAZ mechanosignaling.

Collectively, ECM hydrogels made from natural biopolymers (or derivates thereof) present in the JCT TM region provide a valuable tool for mechanistic investigations of 2D and 3D TM cell-ECM interactions in glaucoma. Disease-associated biochemical (e.g., glucocorticoids, TGFβ2), biomechanical (i.e., ECM crosslinking/stiffening), or cellular (i.e., patient-derived glaucomatous TM cells) stressors provide additional opportunities to investigate nuances in TM cell mechanobiology that may otherwise go unnoticed when relying exclusively on traditional culture substrates. Limitations to the use of ECM hydrogels include the reductionist nature of the hydrogel design (like ECM scaffolds) that cannot completely simulate the native extracellular milieu and the possible variability in photocrosslinking conditions. Differences in cell source (see above) and culture conditions may introduce additional inconsistencies, and the integration of ECM hydrogels into perfusion bioreactors/microfluidic chips for studies under dynamic flow is far from trivial.

3. ECM biomaterials to study SC cell biology

The SC inner wall endothelium is supported by a discontinuous basal lamina and numerous cell-cell contacts with the TM [2]. Despite their close functional interdependence in maintaining outflow homeostasis, there is a significant gap in our understanding of SC cell biology compared to TM cells. This is largely because SC cell isolation is very challenging technically, limiting widespread use of cultured cells for mechanistic studies. In the following section, we discuss the use of 3D ECM-based models to investigate human SC cell (patho)biology.

3.1. ECM hydrogels

As mentioned above, a key feature of ECM hydrogels is the ability to culture cells both encapsulated within and seeded atop the 3D biopolymer network while not requiring adhesive protein coatings compared to other commonly-used culture substrates such as polyacrylamide or other synthetic hydrogels.

Li et al. [34] used their collagen/hyaluronic acid/elastin-like polypeptide ECM hydrogel that mimics the underlying JCT TM region (with collagen type I also serving as a component of the SC inner wall basal lamina) to grow SC cells atop. The study investigated the effects of TGFβ2 exposure on regulating SC cell YAP/TAZ mechanosignaling, and whether pharmacologic YAP/TAZ inhibition could prevent SC cells from acquiring a glaucoma-like phenotype. TGFβ2 was shown to induce fibrotic changes and to augment nuclear translocation of YAP/TAZ as a function of F-actin stability. Treatment with clinically-used verteporfin (without light stimulation) effectively blocked the TGFβ2-induced YAP/TAZ hyperactivity and downstream pathological alterations. These data provide new insights into glaucomatous SC cell dysfunction through the lens of YAP/TAZ mechanosignaling. The authors conclude that pharmacological targeting of YAP/TAZ activity may have therapeutic potential for the treatment of ocular hypertensive glaucoma.

3.2. ECM-alginate hybrid hydrogels

Another important characteristic of ECM hydrogels is their modular composition allowing for substitution or addition of components, as needed. To tune hydrogel stiffness independent of ECM makeup and without compromising cell-biomaterial interactions, additional bioinert polymers such as seaweed-derived alginate can be incorporated. Alginates are anionic linear polysaccharide block-polymers. They interlace with the ECM proteins and form an “interpenetrating network” [35]. A key feature of alginate from a materials perspective is its bidirectional responsiveness to crosslinking (= stiffening) using divalent cations such as calcium, and enzymatic degradation (= softening) using specific alginate lyase [36].

In a recent study, Li et al. [37] reported a 3D ECM-alginate hybrid hydrogel that facilitates on-demand and reversible stiffness tuning. They investigated how ECM stiffening modulates YAP/TAZ mechanosignaling in SC cells, and whether targeted interference with YAP/TAZ activity could alleviate SC pathobiology and thus increase *ex vivo* outflow facility in mouse eyes. Calcium-mediated ECM stiffening was shown to induce pathologic YAP/TAZ activation and cytoskeletal reorganization. These effects were completely reversible through alginate lyase-mediated matrix softening in a distinct time-dependent fashion. Pharmacological inhibition of YAP/TAZ activity using verteporfin (without light stimulation) prevented stiffness-induced SC cell dysfunction in terms of cytoskeletal and ECM remodeling. Perfusion of verteporfin increased *ex vivo* outflow function in normal mouse eyes, accompanied by decreased expression of αSMA in the immediate vicinity of the SC inner wall and the filtering TM. This suggests that the stiffness-tunable ECM hydrogel has the potential to improve our understanding of the biomechanical properties of SC inner wall endothelium and its surroundings. The authors conclude that YAP/TAZ are central, yet potentially druggable, regulators of SC cell dysfunction in response to glaucoma-associated ECM stiffening.

Taken together, ECM hydrogels and hybrid variants containing stimulus-responsive secondary biopolymers, in conjunction with relevant disease-associated stressors (see Section 2.4.), provide a valuable tool for mechanistic investigations of SC cell-ECM interactions in glaucoma. Limitations to this approach are the same as for TM cell-focused investigations discussed above, compounded by the relative scarcity of normal SC cells with patient-derived glaucomatous SC cells being even more exclusive.

4. Future directions

The studies highlighted in this review have enabled substantial progress in our understanding of TM cell-ECM interactions in both healthy and glaucomatous states. The “cross-pollination” of techniques and approaches to studying SC cell behavior is still in the early stages; yet, with clear potential for uncovering further mechanistic underpinnings of their unique biology. All studies herein have focused on either TM cells or SC cells, thereby disregarding their physiological

interaction that is essential to the regulation of outflow function and intraocular pressure homeostasis.

Consequently, the creation of a more holistic TM/SC cell co-culture systems centered around suitable ECM biomaterials has the potential to considerably advance our understanding of the complex interplay between TM and SC cells in the outflow tract, as well as their responses to dynamic changes in the shared ECM milieu under physiologic and pathologic conditions. Inspired by recent high-resolution electron microscopy studies of the TM/SC inner wall interface (Fig. 3A), we have begun the development and characterization of a novel TM/SC cell co-culture ECM hydrogel [38] (Fig. 3B) built around the original photo-crosslinkable ECM hydrogel [29].

Integration of TM/SC cell co-culture ECM biomaterials into microfluidic chips for perfusion studies under dynamic flow have the clear potential to further expand their utility and relevance by simulating the *in vivo* milieu of the conventional outflow tract in ways otherwise not possible with current model systems. This would also open the door to investigating the detailed mechanisms underlying "segmental outflow" regulation by using TM cells derived from these specific anatomical regions [40]. It would be worthwhile to consider adding distal parts of the outflow tract (e.g., SC outer wall endothelium, collector channels) – a relatively understudied portion of the conventional outflow pathway. Moreover, the role of tissue-resident or infiltrating immune cells in modulating the local outflow tissue microenvironment in response to disease-associated stressors could be explored on such a chip.

Given the demanding biomechanical environment in the outflow tissues, investigations of additional mechanobiology pathways (e.g., Piezo1, TRPV4) could further enhance our understanding of the possible contributions of biomechanics to outflow tissue dysfunction in glaucoma. Subjecting the TM/SC cell-laden 3D ECM biomaterials to mechanical stretch would facilitate further interrogations of the role of tensile strain on cellular (patho)biology. Along the same lines, recent evidence from other research fields suggest that chromatin remodeling and epigenetic alterations may play a key role in the cellular response to mechanical cues; this is an intriguing new avenue to dissect the molecular basis of ocular hypertensive glaucoma. Lastly, future studies on cell-cell and cell-ECM interactions in glaucoma could make better use of high-throughput omics techniques (i.e., genomics, transcriptomics, proteomics, lipidomics) to help identify key molecular pathways and possible druggable targets.

These future research directions, requiring collaborations between the bioengineering, basic, translational, and clinical research fields, could (i) enable comprehensive mechanistic studies to identify the causal mechanism(s) underlying outflow dysfunction, and (ii) facilitate the screening and development of novel ocular hypertension/glaucoma drugs.

5. Conclusions

The complex interplay between TM and SC cells with their surrounding ECM in the conventional outflow pathway governs the tissues' critical role in maintaining normal intraocular pressure and thereby overall eye health. This review highlights the importance of ECM biomaterials and their utility in investigating TM and SC cell biology under normal and simulated glaucoma-like conditions. The discussed advancements in the development and application of cell-derived ECM, ECM scaffolds, Matrigel, and ECM hydrogels provide, for the first time, a complete overview of state-of-the-art bioengineered ECM platforms for human outflow cell biology modeling, as well as showcase intriguing research avenues dissecting specific cellular and molecular processes with translational potential.

CRediT authorship contribution statement

Souvik Ghosh: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Samuel Herberg:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization.

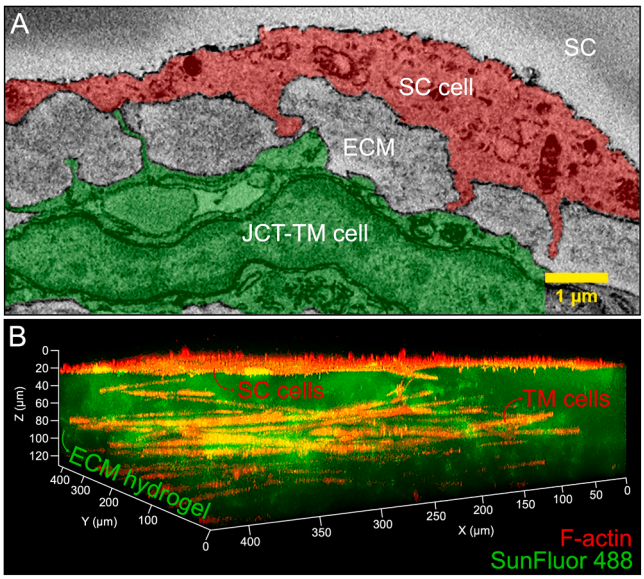

Fig. 3. TM / SC interface architecture. (A) Serial block-face scanning electron micrograph showing the interface between a SC inner wall cell and a JCT-TM cell and their ECM (reproduced and adapted from [39] with permission from Association for Research in Vision and Ophthalmology under Creative Commons license). (B) Engineered TM/SC cell co-culture ECM hydrogel makeup by confocal fluorescence microscopy showing homogenously distributed TM cells within the 3D ECM hydrogel at day 7, with SC cells forming a continuous layer atop the fluorescent hydrogel.

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.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bbiosy.2024.100091.

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