Review Article

Scleral remodelling in myopia and its manipulation: a review of recent advances in scleral strengthening and myopia control

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors: (VII) Final approval of manuscript: All authors.

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Abstract: The biological mechanisms of eye growth and refractive development are increasingly well characterised, a result of many careful studies that have been carried out over many years. As the outer coat of the eye, the sclera has the ultimate impact on the restraint or facilitation of eye growth, thus any changes in its biochemistry, ultrastructure, gross morphology and/or biomechanical properties are critical in refractive error development and, in particular, the development of myopia. The current review briefly revisits our basic understanding of the structure and biomechanics of the sclera and how these are regulated and modified during eye growth and myopia development. The review then applies this knowledge in considering recent advances in our understanding of how the mechanisms of scleral remodelling may be manipulated or controlled, in order to constrain eye growth and limit the development of myopia, in particular the higher degrees of myopia that lead to vision loss and blindness. In doing so, the review specifically considers recent approaches to the strengthening of the sclera, through collagen cross-linking, scleral transplantation, implantation or injection of biomaterials, or the direct therapeutic targeting and manipulation of the biochemical mechanisms known to be involved in myopia development. These latest approaches to the control of scleral changes in myopia are, where possible, placed in the context of our understanding of scleral biology, in order to bring a more complete understanding of current and future therapeutic interventions in myopia, and their consequences.

Keywords: Myopia; sclera; myopia control; scleral strengthening

Received: 01 August 2017; Accepted: 05 January 2018; Published: 21 January 2018.
doi: 10.21037/aes.2018.01.04
View this article at: http://dx.doi.org/10.21037/aes.2018.01.04

Introduction

The sclera is a specialised connective tissue that accounts for the majority of the surface area of the outer coat of the eye. Any change in the size of the eye must therefore be facilitated by changes in the tissue volume and/or surface area of the sclera. Given that axial eye size is the major determinant of refractive error (1), researchers in the field of ocular and refractive development have long been interested in the sclera. In particular, much effort has been devoted to understanding its structure, how its structure changes to facilitate changes in eye size, what factors impact or control these changes and whether the scleral changes are active or passive. More recently the biomolecular and genetic mediators that regulate and facilitate changes in scleral structure have been of particular interest.

Perhaps the main stimulus for the interest in the sclera has been its role in the development of the ocular refractive condition myopia, more specifically because: (I) Myopia has become a major socioeconomic, and therefore major public health, issue around the world (2); and (II) high degrees of myopia are associated with particularly large eyes, in which

stresses on the delicate internal ocular structures result in irreversible damage and, ultimately, blindness (3). It follows that efforts to understand the process whereby the sclera allows the eye to grow excessively have an end goal of applying that knowledge to intervene in the ocular growth mechanisms, ultimately preventing vision loss and reducing the socioeconomic burden of the management of myopia.

Since the earliest research into human myopia, it has been understood that the sclera thins significantly, particularly at the posterior pole of the eye, as the myopia develops (4). Thus, most experimental and clinical approaches to intervening in myopia development either seek to slow, or reverse, the process of scleral thinning (5), or, alternatively, to reinforce the sclera (6), which has become weakened as a result of the thinning. Major advances in this field have been possible over the years through the development of animal models of myopia (7), which allow investigation of the biological mechanisms underlying myopia development, something not usually achievable using post mortem human tissue. In particular, animal models have allowed us to progress our understanding of the mechanisms of scleral remodelling, during myopia development and progression, and have also facilitated the demonstration of methodologies through which scleral remodelling may be slowed, arrested or, in some cases, reversed (8). This review will therefore consider the most recent advances in our understanding of the process of scleral remodelling in myopic eyes, as well as the latest advances in controlling this remodelling and in reinforcing the weakened sclera.

Scleral morphology and underlying biology

The sclera is a dense, irregular connective tissue, consisting of a highly organised and tightly regulated collagenous extracellular matrix. In mammals, it accounts for close to 85% of the surface area of the eye, interrupted only by the cornea anteriorly, and by the entry of the optic nerve into the eye posteriorly (9). The mammalian sclera is a fibrous extracellular matrix, whereas in birds, and other vertebrates, more complex scleral structures are seen, often constituting a thinner fibrous layer, similar to that in mammals, with a thicker, cartilaginous layer that provides the majority of the tensile strength of the structure (5). This review will concentrate largely on the fibrous, mammalian structure that best reflects the structure of the human sclera, however, there will also be reference to studies in avian models of myopia, which also provide much useful information due to the similarity in structure between the thin fibrous layer and the mammalian sclera.

The scleral extracellular matrix comprises largely parallel, but interwoven, bundles of collagen fibrils (10), that, although apparently random in orientation throughout most of the scleral structure, exhibit local areas of directional selectivity, consistent, presumably, with local tensile requirements, such as in the region of attachment of the extraocular muscles (9). These collagenous bundles are typically found to vary in thickness between the inner and outer aspects of the sclera (10). More recent studies suggest that, tangentially, these bundles display a sinusoidal 'waviness', or crimping, in the outer sclera, when compared to the less obvious crimping in the inner sclera (11). In addition, these fibre bundles, which are populated primarily by collagen fibrils that vary in cross sectional diameter between 28 and 280 nm, typically exhibit a reduced average diameter of fibrils in the inner, compared to the outer, sclera (12).

At a molecular level, the scleral collagen fibrils are primarily comprised of type I collagen molecules, interspersed with smaller amounts of at least 11 other collagen types that variously fulfil roles in maintaining structure, regulating fibril size and mediating inter-fibril interactions (13). Although collagenous proteins are known to account for in excess of 80% of the mammalian scleral dry weight (14), there are a number of other important proteinaceous and polysaccharide molecules that reside in the scleral matrix and are of great importance to scleral homeostasis. The scleral matrix contains a range of both small and large proteoglycan molecules that also contribute to fibril structure, mediate interactions between fibrils and control hydration of the scleral matrix (15). Although less is written about this aspect of scleral structure, the collagen fibre bundles are loosely contained within microfibrillar sheaths of small collagen fibrils and elastic fibres (10), and the inter-bundle spaces contain shorter elastin fibrils (11) and, presumably, fluid-filled voids.

The inter-bundle and inter-fibrillar space contains many enzymes and regulators that are of great importance to the structure and function of the scleral extracellular matrix. These include a range of enzymes that control the remodelling of the matrix, such as collagen degrading enzymes and regulators from the matrix metalloproteinase and tissue inhibitor of metalloproteinase families (16), and a range of cytokines and other signalling molecules that control expression of the components of the extracellular matrix, including members of the transforming growth factor-beta (TGF-β) family (17). The fibroblast is the

resident cell of the scleral extracellular matrix and, therefore, responsible for the production, organisation and regulation of scleral matrix structure. A significant proportion of the scleral fibroblast population, however, comprises fully differentiated myofibroblasts (18) which, through an alpha smooth muscle actin (α-SMA)-rich cytoskeleton, bridged to the extracellular matrix through integrin adhesion molecules (19), have the potential to contribute to biomechanical regulation within the sclera.

Given the obvious importance of the sclera to excessive eye growth in myopia, it is no surprise that there has been extensive investigation of each of the elements described above and, in particular, how they change in the myopic eye. These studies have been carried out directly, through the use of animal models of myopia, and indirectly, through observations of post-mortem scleral tissue from human myopes.

Morphological and biochemical scleral changes in myopia

The defining feature of the sclera in a highly myopic eye is that it is thinned significantly, with the thickness sometimes approaching half that of the sclera in an emmetropic eye (4). Excessive degrees of scleral thinning correlate with the posterior staphyloma sometimes reported in human high myopia and with the degree of myopia present in these individuals (20). However, studies in animal models of myopia tell us that a more general thinning of the posterior sclera is likely a feature of all myopia development (12). This gross morphological change in the sclera is, at an ultrastructural level, associated with a preponderance of smaller diameter collagen fibrils in the scleral matrix, leading to a reduction in the gradient in average fibril diameter between the inner and outer sclera. There are also slightly fewer collagen fibre bundles across the entire scleral thickness in myopic eyes, with those bundles also being thinner (12). It is perhaps unsurprising, therefore, that a defined area of scleral tissue from a myopic eye has a lesser dry weight than a similar area of tissue from an emmetropic eye. However, animal models of myopia have also shown that this scleral thinning and tissue loss is not simply a local phenomenon as there is also a reduction in overall scleral dry weight, demonstrating that active loss of tissue occurs as myopia develops, rather than just a simple redistribution of the tissue as the eye enlarges (8).

Although studies in post mortem, highly myopic human eyes have confirmed that the active scleral tissue loss is a result of collagen and, to a lesser degree, mucopolysaccharide depletion (21), animal models have shown this to be a complex process, involving both accelerated scleral matrix degradation and slowed production of new extracellular matrix as an eye becomes myopic (13). The process and causes of this matrix degradation are well documented, with increased production and activation of key matrix metalloproteinases (MMPs) (16,22) associated with decreased activity of their regulators (23), the tissue inhibitors of MMPs (TIMPs), being key contributors. Reduced production of new extracellular matrix manifests as lower levels of collagen synthesis, in particular type I collagen (13), and reduced production of proteoglycans and their glycosaminoglycan (GAG) side chains (8). However, this is also a complex process, in that it creates apparent stoichiometric imbalance between the molecular components of new matrix as it is laid down, which has the potential to contribute to the differences in fibril morphology and matrix structure observed at the morphological level. In particular, there is evidence to suggest that the general reductions in extracellular matrix component synthesis result in an overrepresentation of key factors that influence collagen fibril diameter, such as type V collagen (13) and the smaller proteoglycans (24), presumably contributing to the increased prevalence of smaller collagen fibrils. Demonstrable changes in the polysaccharide profile of the scleral matrix, for example through the reduced accumulation of glycosaminoglycans (25), also lead to the hypothesis that scleral hydration is lessened in the myopic eye. Overall, the changes discussed suggest that, as myopia develops, the scleral matrix becomes a thinner, less rigid biomaterial that is increasingly susceptible to the *in vivo* physiological forces it experiences.

Scleral fibroblasts and myofibroblasts are the orchestrators of the changes in the scleral extracellular matrix of myopic eyes, and are presumed to be driving this change in response to biochemical signals emanating locally, from the retina, in response to specific information contained in the image projected onto the retinal photoreceptors and processed in subsequent retinal layers (26). However, studies in animal models suggest there are also secondary effects on the scleral cells as the matrix changes around them, resulting in altered local stresses that impact cell behaviour. Specifically, *in vitro* studies suggest that increased stresses on fibroblasts within the weakened extracellular matrix, in conjunction with an environment in which levels of matrix-stimulating cytokines, such as TGF-β, are reduced, results in an increase in the number of

cells that transdifferentiate into myofibroblasts (27). This occurs despite evidence that the cells appear to reduce the extent of their adhesion to the extracellular matrix, through reduced integrin production (28) and, presumably, reflects attempts to both regulate the local stress levels experienced by cells and also maintain the integrity of the surrounding matrix.

Given the structural and biochemical changes that occur in the sclera of eyes developing myopia, and the implications of those changes, it is unsurprising that the mechanical properties of the sclera are of particular interest in myopic eyes.

Biomechanical properties of the sclera in myopia

The discovery of a significantly thinned sclera in highly myopic eyes led to the early realisation that the biomechanical properties of the sclera must also be compromised. Subsequent characterisation of these biomechanical properties in post mortem human eyes with high myopia revealed that this was indeed the case. Studies revealed that whereas the sclera from 'normal' emmetropic eyes tended to show regional variations in the degree of stiffness, with sclera from posterior regions of the eye having a lower Young's (elastic) modulus, thus being less stiff, than equatorial or anterior regions (29), scleral tissue from these same regions of myopic eyes showed yet lower stiffness values, indicating that the sclera in myopic eyes may be more extensible under the *in vivo* load profiles experienced by scleral tissue (30).

Given that the sclera is a viscoelastic biological tissue, subsequent studies have determined its properties in response to forces that mimic the eye's physiological load profile, suggesting that the structural changes in the sclera of myopic eyes make them more susceptible to distending, and therefore enlarging, when exposed to the normal forces of intraocular pressure over time (31). This tendency of scleral tissue in myopic eyes to creep under physiological loads suggests that the biochemical factors underlying scleral remodelling and ultrastructural change in eyes developing myopia lead to a consequent biomechanical weakness in scleral tissue which, over time, results in ocular elongation and myopia development. In support of this hypothesis, studies have shown a correlation between the creep properties of the posterior sclera from myopic eyes and the degree of ocular elongation and myopia developed in that eye (31).

Scleral collagen content has a major effect on the biomechanical properties of the sclera, with fibril diameter playing an important role in this (12), while the relative proportions of the different collagen types present in the sclera also have an impact (13). The number of cross-links between collagen fibre bundles also modulate the scleral biomechanics, with increasing age and cross-link numbers leading to a stiffer sclera (32). Proteoglycan concentrations change throughout life, and are also likely to be at least partly responsible for the age-related changes in scleral biomechanical properties (15). A whole range of other growth factors and signalling cascades are involved in the maintenance of the scleral tissue, thereby impacting the biomechanical behaviour of the sclera (33). Given the scleral biochemical changes reported during the development of myopia, it follows that collagen and proteoglycan profile changes and modifications to collagen cross-linking are likely key factors in the measured biomechanical changes in the sclera.

It is now well accepted that the biomechanical properties of the sclera are closely related to its ultrastructural and biochemical makeup, and that specific changes in scleral biochemistry promote ocular enlargement and myopia. It is no accident, therefore, that attempts to arrest the excessive growth of the eye are either aimed at strengthening the extracellular matrix, or at reversing the biochemical changes that drive the mechanical weakness in the myopic eye.

Approaches to scleral strengthening in myopia

A number of different strategies have been employed in an attempt to prevent the progression of myopia through scleral strengthening, each targeting a different aspect of the factors governing scleral biomechanical properties.

Posterior scleral reinforcement (PSR)

PSR surgery was first proposed by Shevelev in 1930, and has a sporadic history of use in the management of high myopia (34). The aim of PSR is to act as a buckle, improving the biomechanical properties of the myopic sclera. Due to the highly invasive nature of the procedure, it has typically only been performed in patients with the highest levels of pathological myopia, frequently with staphyloma formation and/or myopic macular detachment. The various techniques of PSR developed over the years, using donor tissue or synthetic materials, have had various levels of success. The most recent proponents of PSR have been from Russia and China. Biomechanical shortcomings

of the earlier PSR surgeries were hypothesised to be the result of the application of too little force to the posterior aspect of the globe, with the application of appropriately tensioned scleral buckles having been shown to effectively control axial myopia progression (35). Long-term reductions in axial length and refractive progression have been seen using both human donor sclera (6,36) and dura mater (37) as the reinforcement material. Only minor effects of PSR, however, have been observed in younger patients with high myopia (38). In all cases, PSR is only used for the management of high, pathological myopia, and not for controlling the excessive axial elongation associated with lower levels of (physiological) myopia.

Although PSR is typically used in cases of pathological myopia, the anatomical and biomechanical changes that occur in the sclera as a result of PSR may also provide insights into the development of lower levels of (physiological) myopia. Few studies have looked at the tissue response to PSR, and only in animal models to date. The use of synthetic reinforcement materials in rabbit sclera leads to an inflammatory wound-healing response and type III collagen granulation tissue deposition, which is eventually replaced by type I collagen (39). A similar response, of inflammatory reaction followed by fibrosis, also occurs when human donor sclera is used to reinforce rabbit sclera (40). Both the elastic modulus of the sclera and the hydroxyproline content were reduced in the area of reinforced sclera following the surgery, but returned to physiological levels over 9 months (40). It appears that the longer the reinforcement is in place, the greater the increase in scleral elastic modulus that occurs, and that this increase in reinforced elastic modulus is not directly related to the modulus of the reinforcing material (41). Scleral fibroblasts in the area of reinforcement also show altered responses to mechanical stimulation compared to control tissue (42-44). Viscoelasticity of individual scleral fibroblasts in the host sclera was reduced following PSR, but fibroblasts in the fusion zone between the host and donor tissue showed significantly increased viscoelastic properties (42). PSR results in a reduction in MMP-2 in the host tissue compared to normal controls, with the most significant reduction in the transition zone between the host and donor tissues (44). Cyclic stretching of the fibroblasts can further reduce these MMP-2 levels (45). TGF-β1 and FGF-2 levels in the transition zone between host and donor tissue are also significantly increased via mechanical stretching, suggesting enhanced fusion and thickening of the PSR region occurs under mechanical forces. Taken together, these findings suggest that the increasing scleral elastic modulus seen following PSR is a function of induced tissue remodelling beneath the donor tissue that is the reverse of that seen during the development of myopia. The mechanism whereby these altered scleral responses occur following PSR have yet to be fully elucidated, but targeting of the signalling cascade itself may prove equally useful in effecting similar changes in progressing myopic eyes without the need for surgical grafting.

Collagen cross-linking

While PSR has been practised for decades, more recent attempts at strengthening the posterior sclera to prevent myopia progression have focused on scleral collagen crosslinking. Collagen cross-linking has been successfully employed over the past two decades for strengthening the cornea in cases of ectasia, typically in keratoconus (46). The collagen in the sclera naturally contains cross-links, and shows a change in the type (14) and an increase in the number of cross-links with age (47). This is possibly associated with a commensurate increase in scleral stiffness with age (32). When scleral collagen cross-linking is inhibited the degree of experimental myopia that develops is significantly increased (48), highlighting the importance of endogenous scleral collagen cross-links in refractive error development.

In corneal cross-linking, the typical process involves a photo-inducer (riboflavin) and a light source [ultraviolet A (UVA)] to induce the formation of cross-links (46). A number of studies have used animal models to explore the feasibility and myopia inhibiting effect of riboflavin and UVA or blue light cross-linking of the sclera (34). Recently, experimental myopia progression has successfully been prevented by scleral cross-linking *in vivo* in form deprived rabbits (49), with short-term success also reported in negative lens-wearing guinea pigs (50). The *in vivo* cross-linking procedure did not prevent the myopia-induced reduction in collagen fibre bundle numbers, but the fibre bundles were denser, more regularly distributed, and showed some skew towards thicker fibre bundles (50). In eyes treated with scleral cross-linking, but no myopia induction, an inhibition of eye growth is present, further demonstrating the role of scleral cross-links on eye growth cessation (51). In contrast to myopic eyes, when scleral cross-linking is performed *in vivo* without myopia induction the scleral collagen fibre bundles also become skewed towards smaller diameters (52) and increased variability in

fibre bundle composition (53). Biomechanically, the cross-linked sclera shows increased elastic and viscous modulus (53-55), and increased stiffness (53). When human sclera is cross-linked *in vitro*, the response is similar to that observed *in vivo*, with a skew towards thicker collagen fibril diameters and an overall increase in spread of fibril diameters (55,56). Young's modulus increases with increasing intensity of the cross-linking light source in both human (57) and rabbit sclera (58). This effect, however, plateaus, with a decrease in Young's modulus seen with further intensity increases (57), or no biomechanical effect seen if irradiation levels are insufficient (59).

The varied response of the sclera to differing irradiation intensities makes determination of the appropriate level of cross-linking for a given level of myopia and scleral collagen content a challenge. Increasing levels of irradiation provide, in most cases, greater scleral stiffening, but increasing irradiation levels increase the risk of complications. Scleral inflammation (58), collagen destruction or disorganisation (52,53,55), reduction in dark-adapted electroretinogram (ERG) (54), and retinal cell death and layer disruption (51-54,58) have all been observed following cross-linking procedures, particularly with higher irradiance levels. Given that the biomechanical changes required to prevent myopia progression require higher irradiances to achieve, the potential for serious side effects to occur with current procedures warrants careful further investigation before starting human cross-linking trials. Further concern has been expressed about the impact of the cross-linking process on scleral myofibroblasts and vasculature (60), issues which will also need to be resolved if this is to become a viable treatment strategy.

The process of scleral cross-linking, wherein riboflavin and UVA or blue light needs to be delivered to the surface of the sclera for extended periods of time, make this highly invasive procedure impractical for the control of myopia in the general population. Some of the difficulties involved in delivering light to the posterior surface of the sclera may be overcome with the development of flexible optical waveguides to improve the light delivery performance (61) or multimode optical fibres for riboflavin and UVA delivery in a minimally invasive manner (62). A different approach to the traditional cross-linking technique is the use of other cross-linking agents that work without the need for arduous, potentially toxic, activation processes. Genipin and glyceraldehyde have both been shown to increase Young's modulus in the sclera *in vivo* (63,64), with a dose-dependent stiffening of sclera demonstrated *in vitro* (65). Genipin has been shown to effectively prevent axial elongation and myopia development, through form deprivation, in guinea pigs, showing an increase in the scleral Young's modulus accompanied by increased collagen fibril diameter and decreased fibril density (66). Interestingly, however, while glyceraldehyde increases scleral stress and Young's modulus, as expected from cross-linking, it showed no significant effect in preventing myopia development (64). Other agents that have been investigated for enhancing scleral collagen cross-linking that may yet prove beneficial in preventing myopia development include methylglyoxal (65) and a range of formaldehyde releasers including sodium hydroxymethylglycinate (67,68).

While the use of these novel forms of scleral cross-linking are still in their infancy in terms of myopia control, it is important to think of the potential long-term side effects, over and above those immediate histological effects mentioned already. Scleral cross-linking with glyceraldehyde in mice has been shown to have no impact on retinal histology or ERG function while stiffening the sclera, however there was accelerated retinal ganglion cell loss when intraocular pressure was increased as a model of glaucoma development (69). While this increase in glaucomatous damage with scleral cross-linking may be limited to the use of glyceraldehyde as the agent, it may also be relevant to other forms of scleral cross-linking for myopia prevention and should be taken into consideration, given the epidemiological link between myopia and glaucoma (70).

Other physical reinforcements

Sub-Tenon's injections or implants using a range of materials have been trialled as a means to strengthen the sclera and prevent myopia progression (71-74). Scleral strengthening injections in rabbit eyes resulted in granulomatous inflammation that was slowly replaced by new collagen (71), which mirrors the effects of synthetic band scleral reinforcement (39). The procedure also appears to be a viable option for reducing highly progressive myopia in human eyes, with maintenance of a stable refractive error in around 50% of patients by 4–5 years post-injection (71). Sub-Tenon's injection of a hydrogel comprising acrylated hyaluronic acid also successfully prevented the development of experimental myopia in guinea pigs, although, interestingly, eyes injected with a control vehicle containing only buffer also displayed the same inhibition of myopia progression (72). In these cases, there was cellular

infiltration of the hydrogel implant, and thickening of Tenon's capsule for both the implant and control injections, suggestive of the early stages of an inflammatory process. In chicks, however, sub-Tenon's reinforcing injections, while leading to scleral thickening, did not alter the growth of the eye (73,74), highlighting different mechanistic actions in mammalian and avian ocular biomechanical growth characteristics.

Another possible myopia management strategy that has been proposed in theoretical terms is the use of stem cell delivery to the sub-scleral space (75). It has been hypothesised that these implanted stem cells might be coaxed into fibroblasts to enhance scleral collagen production, or to act on dopaminergic pathways to modulate myopia development. Human fibroblasts have been transplanted, in a suspension, onto the posterior pole sclera via sub-Tenon's injection in an effort to prevent myopia development (76). Approximately 40% reduction in the form deprivation myopia induced was noted in eyes with the fibroblast transplant. In all eyes with transplanted fibroblasts there was an increase in type I collagen in the sclera surrounding the implant location, and a decrease in fibroblast numbers four weeks after transplantation. Presumably the increased collagen deposition strengthened the sclera to reduce the myopia progression.

Collagen and proteoglycans

A different approach to strengthening of the sclera is to more directly target prevention of the degradation of the tissue that occurs during myopia development, or enhancement of the production of new tissue to counteract the degradation.

Collagen content in the sclera is significantly reduced during myopia development, with thinning of fibrils, loss of a fibre thickness gradient, and overall reduction in scleral thickness (26). The caffeine metabolite 7-methylxanthine, a non-selective adenosine antagonist, has been shown to prevent form deprivation myopia development in guinea pigs (77) and rabbits (78), although its actual mechanism of action remains a matter of speculation. It has also been shown to display a modest effect in controlling lens-induced myopia in chicks (79), and almost entirely prevents lens-induced myopia development in rhesus monkeys (80). In eyes without a stimulus to myopia development, 7-methylxanthine significantly increases posterior scleral collagen fibril diameter and significantly decreases total GAG content (81). The alteration in scleral collagen fibril diameter is the likely mechanism of action for 7-methylxanthine's myopia inhibition, as treatment with it also prevents the loss of collagen fibril gradient typically seen in myopia development (26,77,78). 7-methylxanthine has also been shown to significantly reduce axial elongation and myopia progression in human trials (82,83), with axial growth returning to the same rate as control following cessation of treatment (84). Long-term follow-up, over eight years, shows an approximate 60% reduction in myopia progression with oral 7-methylxanthine treatment (85). Other potential targets in the collagen synthesis pathway, aiming to increase scleral collagen content and strengthening the sclera to prevent myopia development, include the peroxisome proliferator activated receptors (80,86), cyclic adenosine monophosphate (87), cyclic guanosine monophosphate (88), regulator of G-protein signalling 2 (89), and bone morphogenetic proteins (BMP) (90-92).

Conversely, instead of increasing scleral collagen production to strengthen the sclera, the degradation of pre-existing collagen can be slowed through modulation of MMPs and TIMPs. Supplementation of TIMP-2 has been shown to significantly reduce the amount of collagen degradation during myopia development in chicks (5) and tree shrews (23), although a commensurate reduction in the amount of myopia has only been observed in tree shrew. Pirenzepine, an M1 receptor selective antimuscarinic agent that is effective in controlling human myopia progression (93), has been shown to inhibit experimental myopia development through modulation of MMP-2 and TIMP-2 expression (94). Difrarel, an anthocyanin derived from bilberry, also reduces form deprivation myopia via suppression of MMP-2 and enhanced collagen type I expression (95). Anthocyanins derived from blackcurrants have also been shown to prevent lens-induced and form deprivation myopia (96,97), and although the mechanism of action has not been investigated they may be working through a similar pathway to difrarel. Sonic hedgehog (Shh) has been shown to enhance myopia development in mice and guinea pigs, with an inhibitor of the Shh pathway, cyclopamine, blocking myopia development (98,99). The expression of Shh increases during myopia induction, coincident with increased retinal mRNA expression of blue and red opsins, implicating Shh in their modulation during refractive development (100). Enhanced myopia development through the Shh pathway, however, appears to be through increased MMP-2 production (99), offering another potential target for myopia control.

GAG content is reduced in the sclera of myopic eyes, and this correlates with the peak increase in scleral creep associated with myopia development (101). Removal of sulphated GAGs from the sclera, however, differentially increases scleral stiffness in humans (102) and decreases scleral stiffness in pigs (103). Scleral GAG synthesis has been shown to be upregulated by BMP-2 (91) and insulin (104). GAG levels in scleral fibroblasts reduce in the presence of GABA agonists and increase with GABA antagonists (105), possibly through a dual mechanism involving both intra- and extra-scleral GABA receptors. Interestingly atropine, which is one of the most successful treatments found to date for inhibiting the progression of human myopia (106), has been shown to significantly reverse the elevated retinal and scleral GABA transporter 1 levels seen during myopia development (107). The myopia preventing effect of atropine may thus have an endpoint, at least in part, in a reduction in GABA-mediated GAG inhibition. Peroxisome proliferator activator receptor alpha (PPRAα), which has recently been demonstrated to suppress myopia development through increased scleral collagen synthesis (80), has also been shown to inhibit GAG biosynthesis and counteract the stimulatory effects of TGF-β1 on proteoglycan synthesis in other tissues (108). Inhibition of proteoglycan synthesis by β-xyloside has been shown to prevent form deprivation myopia development in chicks (109). The role of proteoglycans and their GAG side chains in myopia development is clearly highly complex representing a complicated balance in expression levels between the different core proteins and the composition of the bound GAG side chains. The manipulation of proteoglycans and GAGs to control scleral biomechanics, and therefore myopia development, although a theoretically promising pathway, has proved minimally successful to date.

Fibroblasts, myofibroblasts and TGF-β

Scleral myofibroblasts, modified fibroblasts with contractile properties endowed through the presence of α-SMA, have been shown to be present in human (18), macaque (18), tree shrew (110), and guinea pig sclera (111). First identified in granulation tissue from healing wounds (112), the contractile properties of these cells (113) provide an interesting target for arresting, or even potentially reversing, myopia development. In order for fibroblasts to transdifferentiate into the myofibroblast phenotype and express α-SMA they must be exposed to both TGF-β (114) and local tissue stress (115).

TGF-β expression is down-regulated in eyes developing myopia, leading to a reduction in collagen synthesis (17). However, in the presence of stress equivalent to that experienced in the sclera, the reduction in TGF-β has been shown to actually increase expression of α-SMA in cultured scleral fibroblasts/myofibroblasts, presumably through reduced collagen synthesis and increased local stress on the cells (27). This implies that multiple activation pathways exist for targeting myofibroblasts in myopia control. One such pathway whereby TGF-β helps to modulate scleral changes in myopia development is the Wnt/β-catenin signalling pathway. It has recently been shown that inhibition of the Wnt/β-catenin pathway by the antagonist DKK-1 in experimental myopia increases type I collagen expression and induces a more orderly arrangement of the collagen, while neutralisation of TGF-β1 further reduces type I collagen expression (116). Furthermore, stimulation of the Wnt pathway, even in the absence of myopiagenic stimuli, results in a myopic shift in refraction (117). The traditional Chinese medicine *Bu Jing Yi Shi* has been shown to prevent form deprivation myopia development in a dose-dependent manner, increasing scleral TGF-β1 and Smad3 levels as well as leading to increased scleral thickness and fibroblast numbers (118). Smad3 is one of a family of effector proteins that help to transfer the TGF-β signal from outside fibroblasts to the nucleus, one of the possible pathways for activating α-SMA and differentiation of myofibroblasts (114). Interestingly Wnt/β-catenin also appears to play a pivotal role in this pathway of α-SMA activation (114).

Cyclosporine A, an immunomodulatory drug, has been shown to increase TGF-β, α-SMA, and type I collagen expression in fibroblasts from some tissues (119,120), although in other tissue types it has inhibitory effects (121,122). Interestingly, cyclosporine A has recently been shown to reduce experimental myopia progression, although whether this was effected through changes in α-SMA expression was not investigated (123). In gingival fibroblasts cyclosporine A enhances the expression of α-SMA and type I collagen through Shh, regulated by TGF-β (119). Atropine has also been shown to reduce inflammatory markers in the sclera while suppressing myopia development (123). This leads to the possibility of an inflammatory link in myopia development. Indeed, one of the earlier theories of the pathogenesis of myopia development was that it resulted from a low-grade inflammation of the retina, choroid, and sclera: "*The inflammation is usually preceded by congestion of the retina and choroid as the result of excessive and improper*

use of the eyes. The inflammation slowly supervenes, but never becomes active. Myopia in growing children is undoubtedly due to this cause" (124). More recently, myopia has been shown to occur with higher prevalence in individuals with systemic inflammatory disorders (123), showing an association with hay fever (125), and negative lens-induced myopia has been linked to an increase in complement factors (126). There are also links between myopia and other inflammatory ocular conditions (127). Immune pathways have also been implemented in refractive development through differential gene expression analyses (128).

Another aspect of cellular activity in the sclera that may have a role in controlling scleral biomechanical changes in myopia is the adhesion of fibroblasts and myofibroblasts to the surrounding extracellular matrix. Integrins, which mediate cell-matrix interactions, are expressed in mammalian sclera (19,129), and show significant decreases in expression during myopia development (28,130). Basic fibroblast growth factor has been shown to increase integrin and collagen type I expression in the sclera while inhibiting myopia development (130).

It should be apparent that the role of fibroblasts and myofibroblasts in the scleral changes and development of myopia is a complex one, with many different growth factors and signalling pathways involved in the process. While this makes determining the pathway(s) responsible for the pathogenesis of myopia difficult, it does provide many potential targets for strengthening the sclera against myopia development that will need to be investigated.

Candidate genes

A lot of research has been undertaken to investigate the gene expression patterns that occur during the development of myopia, and a full examination of the genes involved in myopia development is beyond the scope of this review. Analysis has shown that the candidate genes involved in human myopia development display significant overlap with the candidate genes identified in experimental animal models of myopia, indicating these models remain suitable for studying human myopia development (131). It is clear that a large number of genes are upregulated or downregulated during development of or recovery from myopia, some of them in a bidirectional manner. What remains unclear, given the large number of candidate genes, is which genes should be targeted for investigation as to their role in myopia development, and particularly as to their role in the sclera. Indeed, it is likely that the condition reflects an interaction of many different genes with small expression changes that leads to the myopic phenotype (132), and, of course, that many of the phenotypic changes in the sclera occur downstream, in the signalling cascade, of some of the candidate genes. Recently, microRNAs, small noncoding RNAs that regulate gene expression, have been investigated for their possible involvement in myopia development (133-136). With large numbers of candidate genes for non-pathological myopia identified through various genetic testing protocols, there is no shortage of potential scleral targets for the control of myopia development to be investigated.

Scleral signalling

One aspect that this review has not yet addressed is how the changes that occur during myopia development are communicated to the sclera. Numerous studies have shown that there is local control of ocular growth, and that the retina detects and responds to the sign of defocus (137). There is evidence that this is modulated, at least in part, by changes in choroidal thickness, and that mediators released by the choroid may signal the sclera to modify its growth parameters (138). There are many potential candidates for the retinal-choroidal signal, the discussion of which falls outside the scope of this review, except to acknowledge that, once isolated, these pathways will provide another, upstream, target for modulating the biochemical, and therefore biomechanical, behaviour of the sclera during myopia development.

Summary

The changes that occur in the sclera during myopia development represent a complex interaction between tissue remodelling, synthesis, and degradation, the upshot of which is a reduction in scleral stiffness and increased extensibility of the posterior sclera. A number of different strategies have been employed to strengthen the sclera and prevent the progression of myopia, which can roughly be divided into reinforcement of the weakened scleral tissue, or modulation of the synthesis and degradation pathways. The technique that has been employed to the most success in humans to date is posterior sclera reinforcement, but the highly invasive nature of the technique and variable outcomes has seen this mostly relegated to cases with the highest levels of pathological myopia. Scleral collagen cross-linking represents a promising alternative to PSR.

Unlike corneal cross-linking, which has found much favour in managing corneal ectasias, scleral cross-linking has proven difficult to implement due to access to the posterior sclera, the proximity of other tissues sensitive to UV damage, namely the retina, and the vascular nature of the sclera. Modulation of the production of collagen by 7-methylxanthine, and inhibition of MMP-2 show promise in strengthening the scleral tissue. The targeting of other pathways, however, has had mixed results.

The main challenges involved in strengthening the sclera to prevent myopia development therefore appear twofold. The first being the location of the posterior sclera within the orbit, and the second being the multitude of finely balanced processes that underlie scleral remodelling that we have yet to fully elucidate. The fact that interventions our current understanding of scleral biochemical and biomechanical processes suggest should reinforce the sclera do not work as expected highlights that the development of a feasible and functional scleral stabilisation technique for the prevention of myopia will rely on our understanding of a complex array of tissue interactions.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editors (Ian G. Morgan, Xiaohu Ding and Xinxing Guo) for the series "Managing Myopia in East Asia Myopia Crisis" published in *Annals of Eye Science*. The article has undergone external peer review.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aes.2018.01.04). The series "Managing Myopia in East Asia Myopia Crisis" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/aes.2018.01.04
Cite this article as: Backhouse S, Gentle A. Scleral remodelling in myopia and its manipulation: a review of recent advances in scleral strengthening and myopia control. Ann Eye Sci 2018;3:5.

