Canaloplasty, a recently developed nonpenetrating glaucoma surgical approach, may restore physiological outflow routes in primary open-angle glaucoma with less risk of severe postoperative complications than trabeculectomy. Since the inner wall of Schlemm’s canal (SC) is directly in contact with the trabecular meshwork (TM) for 360° and the catheter device used in canaloplasty allows viscoelastic to be injected into the entire length of SC, canaloplasty might also be used to perform SC/TM-targeted delivery of transgene vectors for glaucoma gene therapy. This hypothesized new method for transgene delivery may give the transgene access to the entire inner wall of SC and the whole juxtacanalicular region of the TM and allow the transgene to be expressed in both the TM and SC without affecting the cornea, iris, and ciliary body. This strategy might have a greater trabecular outflow resistance-decreasing effect than either the genetic or surgical approach alone.

Intolerable intraocular pressure (IOP) for the optic nerve is a major causal risk factor for primary open-angle glaucoma (POAG)-induced progressive optic neuropathy. The intolerable IOP results from abnormally or relatively (e.g., for “normal” pressure POAG) high flow resistance in the juxtacanalicular trabecular meshwork (TM) and/or the adjacent inner wall of Schlemm’s canal (SC), and perhaps collapse of SC. At present, the only effective approach available to treat POAG, including “normal” pressure POAG, is to reduce IOP either pharmacologically or surgically. Although pharmacological approaches are noninvasive and effective generally, suboptimal tolerance, compliance, and effectiveness over time are still challenges. Importantly, pharmacologic therapies require patients to self-administer topical medications daily, often with poor therapeutic adherence. Traditional surgical approaches are designed to create a drainage pathway for aqueous humor to bypass the dysfunctional physiological outflow routes. For instance, trabeculectomy, the gold standard for glaucoma surgery, redirects aqueous humor outflow to the subconjunctival space, which leads to the formation of a filtering bleb. However, although trabeculectomy usually provides successful IOP control, it may induce severe short or long term complications, including bleb leaks, flat anterior chamber (AC) and hypotony, infections, choroidal effusion, cataracts, and maculopathy. Bleb encapsulation or scar formation in the new surgically created aqueous humor outflow pathway may occur and is the main cause of surgical failure. Laser trabeculoplasty may act directly on the TM to decrease trabecular outflow resistance. However, the effect may only last for a limited time period. In recent years, the potential of a permanent outflow resistance-decreasing effect via gene therapy has attracted researchers’ attention. However, safe and efficient TM-targeted transgene delivery methods still await development. In this article, we review the technique/mechanism of the nonpenetrating glaucoma surgery called canaloplasty, and discuss its potential application in glaucoma gene therapy.
Nonpenetrating Canaloplasty

Canaloplasty is an enhancement of the nonpenetrating surgery viscocanalostomy.9,10 Viscocanalostomy involves unroofing of SC by a superficial scleral flap and a deep scleral flap (extended into clear cornea for 0.5 mm to create a Descemet’s window), injection of high viscosity sodium hyaluronate by a finely polished cannula into the ostia of SC on each side, and excision of the deep scleral flap to form an intrascleral space (scleral lake). Following viscocanalostomy, aqueous humor may percolate from the AC through the Descemet’s membrane window into the scleral lake, where it may enter SC through the surgical canal ostia and leave the eye via either the normal collector channels or via the ruptures of the outer wall of SC created by the injected viscoelastic. Ruptures of the inner wall of SC and the adjacent juxtacanalicular connective tissue (JCT) of the TM could also serve as pathways for fluid flow from the AC to SC. Additionally, this surgical approach might also increase uveoscleral outflow, since aqueous humor passing through Descemet’s membrane into the scleral lake can suffuse into the adjacent uveoscleral outflow system.10 Several studies have shown that viscocanalostomy significantly decreases IOP in POAG patients and increases outflow facility in normal nonhuman primates.9–12 However, although a monkey study12 suggested that the injected viscoelastic material remained in the TM for 1 to 2 months following viscocanalostomy, the viscoelastic may not remain in the canal long enough to prevent healing of the surgical ostia and the ruptured regions of the JCT and canal, which may contribute to surgical failure.13 Additionally, septal structures in SC may prevent the injected viscoelastic from expanding the entire canal, so that the ruptures of SC walls by the injected viscoelastic may only occur in proximity to the surgically created ostia.

Development of fine flexible SC catheters14 allows viscocanalostomy to be modified to canaloplasty. Generally, canaloplasty uses the same nonpenetrating surgical technique as that for viscocanalostomy. However, the fine flexible SC catheter with a beacon-lighted tip allows 360° catheterization of SC under the direct visualization. Therefore, the full circumference of SC can be dilated and stretched by injecting viscoelastic through the surgical canal ostia. Additionally, a suture may be pulled back through the canal as the catheter is withdrawn during the viscoelastic injection. The tension on the inner wall of SC and the TM from the tied suture loop in the canal may potentiate and prolong the SC stretch and dilation by the injected viscoelastic, which may increase the rate and duration of the surgically improved natural trabeculocanalicular outflow.14–16 The long term, continuous fluid flow may in return prevent the ruptures of the JCT and SC walls and the openings of SC into the scleral lake from closing during the tissue healing. Although filtering blebs following viscocanalostomy were frequently detected clinically or by ultrasound biomicroscopy in earlier studies, no filtering blebs were detected clinically following canaloplasty and only one out of 20 patients receiving canaloplasty showed a filtering bleb-like structure as detected by optical coherence tomography and ultrasound biomicroscopy in a recent study.17 Therefore, canaloplasty may decrease resistance in the physiological outflow routes. Studies in POAG patients have shown that canaloplasty induces greater IOP reduction than viscocanalostomy18 and carries less risk of severe postoperative complications than trabeculectomy.15,19–21 However, the IOP reduction efficacy of canaloplasty is still smaller than that of trabeculectomy.22,23 Canaloplasty, thus, may be more suitable for patients who have mild optic nerve damage and can tolerate a higher target IOP or for patients where trabeculectomy would have a high probability of early failure.

Potential Application of Canaloplasty in Glaucoma Gene Therapy

With the great progress of molecular genetic technology in the past two decades, it becomes potentially possible to decrease outflow resistance in POAG permanently by adjusting the outflow tissue’s function genetically. A number of genes (e.g., dominant negative Rho or Rho kinase, exoenzyme C3 transferase, caldesmon, matrix metalloproteinases, and specific siRNAs) may be suitable for glaucoma gene therapy targeting the TM.8 Several potential vectors, including nonviral delivery systems (e.g., naked DNA injection, interfering RNA (RNAi), physical methods, and chemical approaches) and viral delivery systems (e.g., herpes simplex viruses, adenovirus, adeno-associated viruses, and lentivirus-), have effectively induced transgene expressions in cultured TM cells and/or the TM of living animal eyes.8 At present, the commonly used method to deliver transgenes/vectors to the TM in vivo is by transcorneal injection into the AC.

Such intracameral injection is a simple procedure, and the delivered transgene/vector is easily brought to
the TM by aqueous humor flow. Additionally, the AC is relatively immunoprivileged, which may result in fewer immune responses to transgenes. Intracameral injection has successfully induced transgene expression in the TM of rats, cats, and monkeys24–29 (Fig. 1). Although low or moderate doses of viral vectors delivered into the AC by intracameral injection are relatively safe, their viral particles often fail to reach the resistance-generating regions of the trabecular outflow pathway (JCT and the inner wall of SC) probably due to interception by uveal and corneoscleral TM cells.31 High doses of viral vectors may induce effective transgene expression in the JCT region of the TM, but they may also induce substantial cell loss (an indication of viral toxicity) in the inner TM,31,32 clinically significant inflammation in the tissues bordering the AC33,34 and unwanted transgene expression in the cornea, iris, and anterior ciliary body28,33 (Fig. 2).

SC is a circular endothelium-lined channel located internal to the corneoscleral limbus and directly in contact with the TM for 360°. Therefore, SC-targeted delivery of the transgene vector in the eye will give the transgene access to the entire inner wall of SC and the whole JCT region of the TM, and may allow the transgene to be expressed in the target cells without affecting the cornea, iris, and ciliary body. SC-targeted delivery may also reduce potential systemic side effects of the transgene vectors by requiring fewer vectors to induce effective expression in the target cells than does intracameral delivery. Direct delivery of balanced salt solution (BSS) containing human matrix metalloprotease-3 (MMP-3) transgene-expressing adenoviral vector to the SC of human donor eyes by viscocanalostomy has induced high transgene expression in the TM and SC endothelium.34 Canaloplasty might further increase transgene expression in...
the TM/SC compared with viscocanalostomy, by allowing the injected vector to reach the entire circumference of SC.

There are also some disadvantages to using canaloplasty to deliver transgenes into the SC. For example, canaloplasty is more difficult than intracameral injection, and likely cannot be performed repeatedly as can intracameral injection. However, canaloplasty is a safe procedure, and may be well suited to vectors that will produce long term transgene expression. Another disadvantage could be that aqueous humor flow from the AC via the TM to SC, driven by the physiological pressure gradient between the two compartments, in living eyes theoretically opposes movement of the injected vector from SC to the TM. However, substantially decreasing IOP by performing a peripheral corneal paracentesis may reverse the pressure gradient between SC and AC, and thereby allow the injected vector to suffuse from SC to the TM. A recent study has semiquantitatively graded suffusion of fluorescein, BSS solution from SC to AC during catheterization for canaloplasty after IOP was lowered below episcleral venous pressure (e.g., <10 mm Hg) by paracentesis in POAG patients. Fluorescein suffusion from SC to AC was classified into 3 grades: (1) poor suffusion with a spread less than 1/8 of the corneal diameter in the AC, (2) moderate suffusion with a spread between 1/8 to 1/4 of the corneal diameter, and (3) extensive suffusion with a spread beyond 1/4 of the corneal diameter (Fig. 3). The study authors believe that grade 1 suggests a highly pathologic TM and that grade 3 indicates a healthy TM. Nevertheless, fluorescein in SC suffused into the AC in all eyes, indicating that the paracentesis-induced temporary reversal of the pressure gradient between SC and AC might facilitate the vector’s suffusion from SC into the TM. Importantly, grade 1 also suggests that the viral vector delivered by canaloplasty might not substantially reach the iris, cornea, and lens in glaucomatous eyes with significantly elevated trabecular outflow resistance. Additionally, setting IOP at a level just below episcleral venous pressure (e.g., ∼9 mm Hg) by controlled paracentesis before intra-SC vector injection in glaucomatous eyes with mild TM pathologic changes may prevent too much vector from entering the AC. On the other hand, extensive suffusion of fluorescein solution from SC to AC does not necessarily mean that a great number of virus–transgene particles will cross the TM and enter the AC under the conditions above. Endothelial cells of the inner wall of SC and

Figure 3. Semiquantitative gradations of fluorescein suffusion/diffusion from SC to AC in the eyes of POAG patients following injection of fluorescein solution into the SC during catheterization of SC for canaloplasty when the pressure gradient between SC and AC was reversed by paracentesis. Poor suffusion/diffusion of fluorescein from SC into the AC (arrow) suggests a highly pathologic TM (A). Moderate suffusion/diffusion suggests moderate permeability of the inner wall of SC and the TM (B). Extensive suffusion/diffusion indicates a healthy TM (C). Modified from a published article with permission from ARVO.
the JCT, which are the target cells for glaucoma gene therapy, may intercept the majority of the virus–transgene particles when viral vector flows from SC towards the AC, as the uveal and corneoscleral TM cells do when viral vector flows from AC to SC following intracameral injection.31

It is unknown how fluorescein suffuses into the TM from SC. However, the naturally existing pores and the canaloplasty-created ruptures of the inner wall of SC might be involved. The endothelial cells of the inner wall have a total of approximately 20,000 pores with diameters of up to 3 μm.35 These pores with adjacent JCT may cause a funneling effect in which aqueous humor from the AC flows preferentially through those regions of the JCT near the pores and then enters SC through the pores.36 This hydrodynamic interaction between the inner wall endothelium and the JCT may lead to a resistance that is larger than the resistance these tissues would generate individually without their proximity to one another.37 However, when the injected vector solution flows from SC to the TM under a reversed pressure gradient, the transgene particles could directly cross all pores and enter the JCT. Retroperfusion (fluid flowing from the limbus/SC to the TM) with glutaraldehyde at zero or negative IOP in enucleated human eyes has confirmed the existence of ostensibly normal pores in inner wall cells under the condition of reversed fluid flow.38 Additionally, the pressure gradient across the JCT and inner wall endothelium may generate mechanical loads on these tissues and in turn affect the tissues' permeability. A previous study has shown that, with increasing IOP, the JCT expands and the inner wall of SC protrudes into the lumen of SC, which may facilitate fluid flow and passage of particulates (e.g., pigment, cellular debris) from AC to SC; whereas, when IOP is reduced below episcleral venous pressure, the JCT and inner wall are compressed together to form a compact structure, which may function as a one-way valve preventing reflux of blood cells and plasma from SC into the AC.39 However, the intra-SC injection-increased canal pressure may “break” the so-called “one-way valve” by pushing the inner wall toward the TM and allow the injected vector to flow into the JCT through opened pores. Particularly, when ruptures are created in the inner wall by the injected viscoelastic during catheterization for cananplasty, the vector flow from SC to the TM may be significantly increased. A study in monkeys has shown that, following viscoanalogostomy, multiple defects were present in the endothelial lining of SC inner and outer walls, and the JCT contained homogeneous material resembling the injected viscoelastic, suggesting that some injected viscoelastic entered the JCT.12 Therefore, in addition to BSS, viscoelastic could be another choice as a solvent for transgene vectors delivered by canaloplasty. Although the diffusional mobility in viscoelastic material may decrease with an increasing radius of the tracer particle, small molecules (and likely small virus– or nonvirus– transgene particles) in the viscoelastic may still diffuse to adjacent target tissues.40–42 Nanoparticles carrying transgene vectors might increase the transgene’s diffusion in the viscoelastic.43 Additionally, biodegradable hydrogels may be the most suitable solvents for transgenes, including large particles (e.g., virus or macromolecular proteins), since these viscoelastic materials are excellent for the preservation of protein structure and function and for the release of biotherapeutics.44 If transgenes are delivered into the JCT with hydrogel by the intra-SC injection, they may be completely released into the target tissue following the hydrogel’s degradation.

Indeed, the paracentesis-reversed pressure gradient between SC and AC may only last for a short period due to aqueous humor formation (e.g., an entire human AC volume could be replaced in 90–100 minutes). However, the transgene vector that has suffused into the angle area following the paracentesis-reversed pressure gradient will flow back into the TM when the pressure gradient returns to its physiological status, which increases the exposure time of the TM to the vector. Thus, the vector may hit the TM from either side or from both sides. Additionally, the suffused vector in the TM might not be washed away immediately after IOP returns to the preparacentesis level, especially when transgenes are delivered with viscoelastic. The injected viscoelastic material might remain in the TM for 1 to 2 months.12 This duration is long enough by far for the transfection and transduction of the target cells by viral vectors, since these processes may only take at most a few hours,45,46 or perhaps even less than an hour.47 A study in organ-cultured human anterior segments has confirmed that 30 to 60 minute retroperfusion with medium containing adenovirus-lacZ reporter gene, followed by 5 to 7 days of conventional perfusion (fluid flowing from the AC to the TM) with gene-free medium, induced more significant gene expression in cells in the JCT and SC than gene delivery through the AC during conventional perfusion.41

Viral vectors can elicit an immune response, and
are potentially toxic. Compared with viral vectors, nonviral gene delivery systems evoke almost no immune response and are relatively safe. Although the transfection efficiency of the nonviral delivery systems is lower than that of viral vectors, direct delivery of nucleic acid to target sites may improve transgene expression. This suggests that TM/SC targeted nonviral transgene delivery by canaloplasty, for example, using a transgene-containing cationic peptide-coated intracanalicular suture or injecting the nonviral transgene into SC during catheterization, might allow significant transgene expression in the TM or SC.

Finally, gene therapy through canaloplasty might also allow the genetic and surgical canaloplasty approaches to potentiate each other and produce an additive effect on the trabecular outflow resistance. This hypothesis might be tested in enucleated and live monkey eyes using specifically designed fine flexible SC catheters and viral or nonviral vectors containing cytoskeleton-modulating proteins (e.g., exoenzyme C3 transferase or caldesmon).24,28,29,45,49

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