Prospective study on a novel treatment for leaking cystic bleb: Efficacy and safety of collagen crosslinking

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Abstract
Importance: Management of cystic bleb leak is difficult. It would be essential to look for a method to strengthen the original pathological conjunctiva and reverse bleb leak.
Background: To evaluate the long-term efficacy and safety of collagen crosslinking in patients with leaking cystic bleb.
Design: Prospective interventional case series at a university-based hospital.
Participants: Twelve eyes in 12 subjects with late-onset bleb leak from cystic bleb, without indications for prompt surgical interventions were included.
Methods: The subjects underwent crosslinking with 0.1% riboflavin application to bleb surface, followed by ultraviolet irradiation for 30 minutes. The subjects were followed up at baseline and at 1 week, 1 month, 3 months, 6 months post-treatment and then every 6 months afterwards.
Main Outcome Measures: Interval from treatment to cessation of bleb leak, recurrence rate of bleb leak and side effects of treatment.
Results: The mean follow-up after crosslinking was 29.33 ± 12.45 months. Bleb leak subsided in 11 (92%) of 12 patients after a single session of crosslinking, after 1 to 8 weeks (median 3 weeks). Time to leak cessation was significantly correlated with the number of prior glaucoma interventions ($R = .71$, $P = .014$). Bleb wall at 3 months was significantly thicker than at baseline (0.70 ± 0.67 vs 0.81 ± 0.62 mm, $P = .008$). None of the patients experienced any complications.
Conclusions and Relevance: Crosslinking achieves resolution of cystic bleb leak which lasts for at least 12 months, without the need of subsequent surgical interventions. Crosslinking is a simple, non-invasive treatment for bleb leak. It aims to restore the integrity of conjunctiva.

Key Words
crosslinking, cystic bleb, filtering bleb, bleb leak

1 | INTRODUCTION

Conjunctival scarring is the main reason for glaucoma filtration surgery failure. Anti-metabolites such as mitomycin C and 5-fluorouracil (5-FU) are used to prevent conjunctival scarring. However, anti-metabolites may also lead to cystic bleb formation.1-5 This complication usually has a delayed onset postoperatively.3-5 The frequency of the late-onset...
bleb leaks ranges from 1.8% to 10%. In some cases it results in hypotony, blebitis or even the dreadful complication of endophthalmitis, which could result in loss of vision. The mechanism of cystic bleb formation remains unclear. In case of the restriction of aqueous outflow to a small area at the subconjunctival space, the pressure inside the bleb increases that could cause conjunctival epithelial damage. Pathology of the thin bleb wall showed the conjunctival hypocellularity and breakdown in the basement membrane connection.

Treatment of late-onset bleb leak is challenging. Possible treatment modalities include cyanoacrylate glue, fibrin tissue glue, injection of autologous blood, Nd:YAG laser application over fluorescein stained conjunctiva, amniotic membrane transplantation and surgical revision. Surgical revision with bleb excision and conjunctiva advancement is difficult in patients who have received multiple glaucoma surgeries with resultant fragile and scarred conjunctiva. Ophthalmologists should try to preserve healthy conjunctiva for further intraocular pressure (IOP) control interventions, especially in young glaucoma patients. Other inventions, although are less invasive, the effects usually do not last long. Non-incisional treatment for bleb leakage usually has a high recurrence rate of leaking in comparison with surgical treatment. Therefore, there is a need of a less invasive procedure to fill in the gap between current medical and surgical treatments to manage these cases. An ideal management would be to reverse bleb leak while maintaining the integrity of the conjunctiva. We should target at treating the pathology underlying bleb leak by restoring a healthier conjunctiva.

Collagen crosslinking (CXL) is a technique used in strengthening the chemical bonds in cornea with ultraviolet (UV) light and photo-sensitizer. CXL has been proven effective in halting keratoconus progression for at least 12 months in previous studies. It can also be used in corneal ectasia following laser refractive surgery such as laser in situ keratomileusis, which occurred more commonly in adolescence. CXL could stabilize ectasia by halting progressive corneal thinning and recovering the anterior and/or posterior curvature of cornea. CXL using riboflavin and UV light received Food and Drug Administration of United States approval on April 2016. Our pilot study of late-onset bleb leaking published in 2016 showed that cystic bleb leak stopped within 1 month after one session of CXL treatment in five patients, and the effect could be sustained for at least 20 weeks. Since then we have recruited additional patients and followed up the patients for at least 6 months to study the long-term results of CXL. Another retrospective study by Wang and Harasymowycz found that 71% of bleb leaking ceased after one CXL treatment at 1 year. Our group’s study on cattle conjunctiva showed that CXL could increase covalent bonds and reduce permeability of the conjunctiva. It could potentially reverse the pathology of the thin cystic bleb.

As far as we know, there was no prospective study on the long-term effect and safety of CXL on bleb leak. CXL is a non-invasive procedure and can be repeated when necessary. It is safe and rebuilds the structure of conjunctiva. The results may give an insight into the treatment of cystic bleb, which current treatments tend to be more invasive and short-lasting effects.

2 METHODS

This prospective study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. It adhered to the tenets of Declaration of Helsinki. Informed consent was obtained from all subjects.

The methodology has been described in details in our previous paper. Consecutive adult patients with leak over cystic bleb surface, confirmed by Seidel test under slit lamp, were included. Diluted fluorescence was applied to inferior fornix to look for bleb leak initially. If leak could not be demonstrated, wet fluorescence strip would be applied directly on the bleb surface with mild pressure on the globe. All patients with bleb leak eligible to be recruited were referred to a single investigator (B.N.K.C.) for assessment. Patients who were indicated for immediate surgical intervention to stop the bleb leak due to complications related to hypotony, those with shallow anterior chamber compared to the fellow eye and those with blebitis or endophthalmitis were excluded. Preoperative data including Snellen best-corrected visual acuity (BCVA), IOP using Goldmann applanation tonometry, clinical photo for the bleb and anterior segment optical coherence tomography (ASOCT) were collected. The thickness of the bleb wall was evaluated with ASOCT. To identify the same location of cystic bleb in each scan, the following rules were applied: (a) all measurements were taken by a single investigator; (b) the centre of the bleb was scanned at the same orientation at the closest proximity compared to previous scan (with landmarks on the bleb/conjunctiva) during each follow-up; (c) the thickness at the apex of the wall was calibrated and three measurements were taken. The average of three calibrates was used for analysis.

After bleb leak was diagnosed, patients were given topical chloramphenicol eye drop to be applied four times a day. As we expected some bleb leak might resolve spontaneously with time, we did not perform CXL immediately following bleb leak was identified. All subjects were reviewed at least twice after for persistent leak before CXL was performed. As the leak occurred diffusely across the cystic bleb,
bandage contact lens was not attempted as it could not effectively cover the leaking area.

CXL was conducted under local anaesthesia with topical 0.4% oxybuprocaine (Novain, oxybuprocaine hydrochloride 4.0 mg; Agepha Pharma sro, Bratislava, Slovakia). Riboflavin 0.1% (Peschke D by Peschke Trade GmbH, Switzerland) was applied to the cystic bleb surface 1 drop every 3 minutes for 30 minutes, followed by UVA irradiation (CCL-Vario,

**TABLE 1** Summary of all the subjects (n = 12)

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnose</th>
<th>Eye</th>
<th>Date of procedures</th>
<th>Previous glaucoma procedure</th>
<th>T1 (y)</th>
<th>T2 (d)</th>
<th>T3 (wk)</th>
<th>T4 (mo)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>84</td>
<td>POAG</td>
<td>L</td>
<td>August 2014</td>
<td>ExPress shunt + MMC</td>
<td>0.4</td>
<td>4</td>
<td>4</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>84</td>
<td>POAG</td>
<td>L</td>
<td>October 1995</td>
<td>TBx + MMC</td>
<td>9.5</td>
<td>13</td>
<td>1</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>57</td>
<td>POAG</td>
<td>R</td>
<td>March 2005</td>
<td>TBx + MMC; Bleb needling + 5-FU; Bleb needling + MMC; AGV Excision of encapsulated bled + MMC; TSCP; TBx + MMC</td>
<td>9.5</td>
<td>48</td>
<td>8</td>
<td>49</td>
<td>Thin cystic bleb at point of leak, but scarring in surrounding conjunctiva</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>54</td>
<td>Uveitic Glaucoma</td>
<td>R</td>
<td>December 2011</td>
<td>Phaco + PCIOL + TBx + MMC</td>
<td>3</td>
<td>46</td>
<td>1</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>66</td>
<td>POAG</td>
<td>R</td>
<td>May 2008</td>
<td>TBx + MMC</td>
<td>5.5</td>
<td>24</td>
<td>2</td>
<td>31</td>
<td>Profuse leak over cystic bleb surface at baseline</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>66</td>
<td>POAG</td>
<td>R</td>
<td>August 2016</td>
<td>TBx + MMC</td>
<td>0.3</td>
<td>73</td>
<td>1</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>53</td>
<td>POAG</td>
<td>L</td>
<td>September 2012</td>
<td>1st ExPress + MMC; Bleb needling + MMC; Bleb-revision + MMC; 2nd ExPress + MMC</td>
<td>4.5</td>
<td>103</td>
<td>4</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>36</td>
<td>POAG</td>
<td>L</td>
<td>1997 October 2002</td>
<td>TBx; TBx + MMC; Auto-blood injection</td>
<td>9</td>
<td>48</td>
<td>1</td>
<td>23</td>
<td>Leak from limbal edge recurred at 1 mo (but no leak from bleb surface) despite initial cessation of leak at 1 wk. CXL was repeated 4 mo after initial CXL and recurrent leak subsided 8 wk after repeated CXL</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>60</td>
<td>PACG</td>
<td>L</td>
<td>August 1995</td>
<td>TBx + MMC</td>
<td>11</td>
<td>36</td>
<td>1</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>67</td>
<td>Uveitic Glaucoma</td>
<td>R</td>
<td>February 2011 May 2011 December 2011, January 2015 and March 2015</td>
<td>TBx + MMC; Ologen; Phaco + PCIOL + Phaco + PCIOL + bleb needling + MMC; Bleb needling + MMC</td>
<td>6</td>
<td>70</td>
<td>8</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
Peschke Meditrade GmbH) using a power of 3 mW/cm² for 30 minutes, with continued riboflavin application every 3 minutes. The cornea surface was protected from UVA by Merocel (polyvinyl acetate sponge, Medtronic Xomed, Jacksonville, FL, USA) during the entire treatment process. After treatment, patients were followed up weekly until bleb leak resolved. Subsequently they were followed up according to schedule at 1 week, 1 month, 3 months, 6 months and then every 6 months afterwards. Chloramphenicol eye drop was continued four times a day until bleb leak resolved following CXL. The main outcome measures were the interval from CXL treatment to cessation of bleb leak and whether bleb leak recurred. The secondary outcomes included IOP, VA, bleb wall thickness and side effects of CXL.

The data were presented in the form of mean ± SD and analysed using SPSS (version 16.0; SPSS Inc., Chicago, Illinois) and Prism Graphpad (version 6; La Jolla, California). Paired Student t tests were used to assess differences in bleb wall thickness, BCVA, IOP, between the pre- and post-operation of CXL. Univariate linear regression was used to evaluate the relationship between the time of cessation of bleb leak with the number of filtration surgeries and the total number of glaucoma interventions. P value of less than .05 was considered to indicate significance.

3 | RESULTS

Twelve eyes of 12 consecutive patients were recruited between August 2014 and September 2017. All patients included in this series have been followed up for at least 12 months after CXL, 11 of them had follow-up for at least 18 months. The mean follow-up was 29.33 ± 12.45 (range 12-49) months after CXL. The demographic and clinical data are shown in Table 1. Nine patients were diagnosed as primary open-angle glaucoma, two uveitic glaucoma and one primary angle-closure glaucoma. The mean age was 62.38 ± 16.45 years. All patients received at least one glaucoma filtration surgery with mitomycin C 0.4 mg/mL for 3 minutes. The mean time from filtration surgery to the diagnosis of bleb leak was 5.43 ± 3.71 years. All subjects had

![FIGURE 1](A) The slit lamp photos with fluorescence pre- and post-collagen crosslinking (CXL). A. There was prominent leak as in classical Seidel test (black arrow) before treatment (upper photos). Bleb leak subsided at 2 months post repeated CXL (lower photos). B. Diffuse sweating (circled area) at baseline (upper photos). No leaking was found at 3 months post CXL (lower photos)
localized avascular cystic bleb (as shown in Figure 1) and they all had spontaneous diffuse pin-point leak from the surface of the bleb without leakage from the limbal edge. The mean interval from the identification of bleb leak to the performance of CXL was 42.7 ± 21.9 days.

In all patients, bleb leak subsided after a single session of CXL, ranging from 1 to 8 weeks after CXL. The median time was 3 weeks. Among these patients, seven (58.3%) of them had no more leak at 1 week, three (25%) bleb leak subsided at 4 weeks and two (16.7%) subsided 8 weeks after CXL. Both of the patients who required 8 weeks for the leak to subside received multiple glaucoma interventions, including trabeculectomy, repeated trabeculectomy and needling, requiring multiple administrations of anti-metabolites. One patient who received trabeculectomy 11 years ago was found to have no leak at 1 week, but leak recurred at limbal edge only at 1 month follow-up (Figure 1A—upper photos). CXL was repeated for persistent slow leak 4 month after initial CXL. IOP was 10 mm Hg and anterior chamber has all along been stable. After repeated CXL, leaking subsided at 1 week, but then pin-point leaking from limbus recurred at 1 month. No leaking was found from 2 months post repeated CXL onwards with conservative management until the last follow-up at 23 months (Figure 1A—lower photos). However, since most of the bleb leak was only diffuse sweating, which was relatively mild and slow, in most of the cases, we could not demonstrate dilution of fluorescence along the leaking point as in classical positive Seidel test, as shown in Figure 1B.

Time to cessation of bleb leak was significantly correlated with the number of filtration surgeries (linear regression, $R = .61$, $P = .048$), and the total number of glaucoma interventions ($R = .68P = .02$). The results were presented in Figure 2.

Clear margin of bleb wall was identified with ASOCT in nine patients, which enabled us to analyse its thickness. There was a trend of thickening of bleb wall from baseline to 3 months as shown in Figure 3. Bleb wall at 3 months was significantly thicker than at baseline (0.70 ± 0.67 vs 0.81 ± 0.62 mm, paired $t$ test, $P = .008$).

BCVA was stable after CXL throughout the entire follow-up, with a variation within one Snellen line. Only one patient had an improvement in BCVA from 0.1 to 1.0 (Snellen VA), which should be attributed to capsulotomy at 2 months post-CXL. The Snellen VA was converted to logMAR VA for statistical analysis. No significant changes between baseline and all the follow-ups (Figure 4A).

IOP trend was shown in Figure 4B. There was no significant change between baseline and the last follow-up (10.55 ± 4.32 mm Hg vs 13.2 ± 5.58 mm Hg, paired $t$ test, $P = .55$). IOP increased in one patient from 13 to 25 mm Hg at 3 months post-CXL, but decreased to 18 mm Hg with one medication at 6 months. All the other patients were not on anti-glaucoma medications. All except one patient had IOP ≥ 6 mm Hg on last follow-up. One patient had an IOP of 4 mm Hg (hypotony), which was static from baseline to the latest follow-up. None of the patients had hypotony-related complications such as maculopathy or choroidal effusions before and after the procedure, nor did they have any other complications such as corneal haze, anterior chamber inflammation and discomfort.

4 | DISCUSSION

A single session of CXL was successful in halting late-onset bleb leak within 1 to 8 weeks in 92% of our patients without recurrent bleb leak. Only one patient required repeated CXL. However, bleb leak also stopped from 2 months post repeated CXL until the latest follow-up at 23 months. The follow-up period of our current series (29.33 ± 12.45 months [range 12-49 months]) is long enough to suggest that CXL is an effective long-term solution for cystic bleb leak. From our observation, time to cessation of bleb leak is related to the number of prior glaucoma interventions. Those who received only trabeculectomy and MMC for once
tend to have cessation of bleb leak within 1 week following CXL, whereas those with previous multiple glaucoma interventions, and multiple administration of MMC and 5-FU, would take up to 4 weeks before bleb leak subsided.

Cystic bleb wall significantly thickened at 3 months compared to baseline. This might be due to the increase in covalent bonding, and collagen fibres could be laid on the thin conjunctiva as part of the healing process. Nonetheless, more experiments would be required to identify if genuine thickening of conjunctiva occurs following CXL. The observation of bleb wall by in vivo imaging was reported for the first time. ASOCT gives more evidence of the mechanical effect of CXL on conjunctiva in a clinical setting, which we have not tested on our previous animal model. A histopathological study of two cases found a significant increase in conjunctiva epithelial proliferating cells after

**FIGURE 3** Anterior segment optical coherence tomography demonstrating the thickness of bleb wall pre- and post-collagen crosslinking in three subjects (A-C). D, The bleb wall thickness and baseline and follow-up. **Significant increase in bleb wall thickness at 3 months compared to baseline (paired Student *t* tests, *P* < .01)

**FIGURE 4** Best-corrected visual acuity (A) and intraocular pressure (B) at baseline and follow-up
CXL using riboflavin and UVA, which is in line with our clinical observation. There could be remaining fibroblasts and growth-arrested cells around the ischaemic bleb, which stimulated conjunctiva epithelial growth. However, more histological analysis following CXL would be required to give sufficient evidence regarding the beneficial effect of CXL on conjunctiva.

Only one patient required repeated CXL. After the treatment was repeated, complete cessation of bleb leak occurred at 2 months. The pin-point leakage from the limbal edge was probably because during the time of CXL, the cornea was shielded with Merocele, which might block the UVA illumination of the limbal edge especially when the patient moved the eye. Therefore, good co-operation of the patients is important, such that UVA could be applied evenly on the desired area of the cystic bleb leak. When the procedure was repeated, we had assured that the limbal edge was sufficiently illuminated throughout the procedure. The cessation of bleb leak up to 2 months suggests a continuous proliferation of fibroblast and epithelial cells even months following CXL.

IOP remained stable at baseline compared to postoperatively. Similarly, Wang and Harasymowycz reported no significant IOP change at baseline and final visit. There were two patients with low IOP at 4 mm Hg and 6 mm Hg, respectively, before CXL. Their IOP remained the same at the final visits. We assume that there was hyposecretion of aqueous in these patients, which existed for a long time. The balance of aqueous may have been established such that despite no leak was present, IOP remained low. In contrast to surgical intervention, which may lead to subconjunctival scarring that impede filtration and thus may cause IOP elevation, CXL does not result in an increase in IOP. This is particularly important in eyes with advanced glaucoma. BCVA also remained stable following the treatment. None of our patients experienced any other complications including infection, or discomfort.

From our earlier patients, a single session of CXL is effective in resolving bleb leak and sustained for up to 4 years. It is a promising long-term treatment. Bleb leak resolved in all our patients with CXL, without the need of surgical intervention. This simple and effective procedure is particularly valuable in patients with scarred and fragile conjunctiva following filtration surgery.

Limitations of our study include this series are the small number of patients enrolled and not a randomized control trial (RCT). A RCT with more subjects to compare CXL and conventional treatment methods could give stronger evidence regarding its efficacy. Furthermore, in assessing the thickness of bleb wall using ASOCT, we have tried our best to ensure all the measurements of the same subject were taken at the closest proximity to the previous scans (by using some landmarks on the bleb wall/sclera. However, since the locations of the measurement would still need to be done manually, possible errors in the measurement was unavoidable. Another limitation is our study had the different follow-up periods. However, since the follow-up was at least 12 months (all except one had follow-up for at least 18 months) and mean follow-up was 29.33 ± 12.45 months, it should be fair enough to say bleb leak cessation would be maintained for a reasonable period of time.

To conclude, CXL is a safe and effective long-term treatment for late-onset cystic bleb leak on patients who do not require immediate surgical interventions. In some cases, particularly those who have received multiple glaucoma interventions, bleb leak might take up to 8 weeks to subside. We might need to wait for a longer period of time in these patients before claiming CXL is unsuccessful and proceed with surgical intervention. In case of recurrent bleb leak, CXL could be re-attempted, which could potentially achieve resolution of leak without the need of surgical interventions.

**CONFLICT OF INTEREST**
None declared.

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**REFERENCES**


