UC Berkeley

UC Berkeley Previously Published Works

Title

Diffuse lamellar keratitis associated with tabletop autoclave biofilms: case series and review.

Permalink

https://escholarship.org/uc/item/0q736909

Journal

Journal of Cataract & Refractive Surgery, 46(3)

ISSN

0886-3350

Authors

Sorenson, Andrew L
Holland, Simon
Tran, Kathy
et al.

Publication Date

2020-03-01

DOI

10.1097/j.jcrs.0000000000000070

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

ARTICLE

Diffuse lamellar keratitis associated with tabletop autoclave biofilms: case series and review

Andrew L. Sorenson, MD, Simon Holland, MB, FRCS, FRCSC, FRCOphth, Kathy Tran, OD, David J. Evans, PhD, Meng C. Lin, OD, PhD, Nick Mamalis, MD, David F. Chang, MD

Downloaded from http://journals.lww.com/jcrs by fJtK+UJHRnS+MUIXYVAQ4WdpBV872fZl4+Z0COS8lQqlMD7HOHn/quYmhgonnE9mW+Qt5uZPsH/EIOJ4roSIF1MIMbYcoKpnplkDbyLVxjLc31CRoRWE+A== on 03/09/2020

Purpose: To report a diffuse lamellar keratitis (DLK) cluster attributed to autoclave reservoir biofilm and to review the risk and prevention of DLK and toxic anterior segment syndrome (TASS) caused by such biofilms.

Setting: Refractive Surgery Center, University of California, Berkeley.

Design: Observational case–control study and review of literature.

Methods: Eyes were evaluated for DLK following laser in situ keratomileusis (LASIK) over a 5-year period. Multiple changes in surgical and operating room protocols were prompted by a cluster of DLK cases. The autoclave reservoir chamber wall was cultured for microbial contamination. The MEDLINE database was used to identify relevant past publications.

Results: From January 7, 2010, to December 18, 2014, 1115 eyes received LASIK. Between September 2, 2010, and June 11, 2012, 147 eyes of 395 LASIK cases developed DLK (37.2%). Systematic modifications in surgical protocols were unsuccessful in ending the prolonged cluster of DLK cases until the STATIM 2000 autoclave was replaced with a new STATIM autoclave and a reservoir sterilization and surveillance protocol implemented. Over the subsequent 30 months, DLK incidence was reduced to 2.2% (14 DLK cases from 632 total LASIK cases, $P < .0001$). The retired autoclave reservoir chamber wall cultures grew *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex.

Conclusions: Fluid reservoirs of tabletop steam autoclaves can readily develop polymicrobial biofilms harboring microbial pathogens, whose inert molecular byproducts can cause DLK and TASS when introduced to the eye by surgical instruments. Stringent reservoir cleaning and maintenance may significantly reduce this risk by preventing and removing these biofilms.

J Cataract Refract Surg 2020; 46:340–349 Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS

Toxic anterior segment syndrome (TASS) and diffuse lamellar keratitis (DLK) are both anomalous postsurgical inflammatory syndromes that generally follow otherwise uncomplicated ocular surgery.[1–3] The exaggerated inflammatory reaction is often caused by a toxic substance introduced during surgery and usually responds to intensive topical steroid treatment. Because laser in situ keratomileusis (LASIK) typically manifests no intrastromal corneal inflammation, DLK is usually obvious and alarming when it appears.[4] In contrast, TASS may be unrecognized and underreported because mild or moderate cases may appear to be within the normal spectrum of postsurgical inflammation. Severe TASS may be misdiagnosed and treated as culture-negative endophthalmitis.

The DLK and TASS literature reports a long list of potential causative factors that have been associated with specific outbreaks. The risk factors can be subdivided into the following categories: (1) disposable surgical items, (2) reusable surgical items, (3) intraocular and extraocular solutions used during surgery, (4) modifications in surgical techniques, and (5) instrument contamination during sterilization and processing. Among more than 30 potential causes identified in the literature, autoclave reservoir biofilms have received relatively little attention as risk factors for DLK or TASS. However, the ability of biofilms to potentiate innate immunity through the system of pattern recognition receptors (PRRs) has become more widely recognized during the last 15 to 20 years. A widely held

Submitted: July 31, 2019 | Final revision submitted: October 31, 2019 | Accepted: November 17, 2019

From the Department of Ophthalmology (Sorenson), Alta Bates Summit Medical Center, and School of Optometry (Sorenson, Tran, Evans, Lin), University of California, Berkeley, California, USA; Department of Ophthalmology (Holland), University of British Columbia, British Columbia, Vancouver, Canada; College of Pharmacy (Evans), Touro University California, Vallejo, Vision Science Graduate Group (Lin), University of California, Berkeley, California, and Moran Eye Center (Mamalis), University of Utah Medical Center, Salt Lake City, Utah, and Altos Eye Physicians (Chang), Los Altos, California, USA.

Drs. Sorenson, Holland, Mamalis, and Chang are members of the ASCRS/AAO/OOSS Ophthalmic Instrument Cleaning and Sterilization Task Force.

Stephanie McGovern, RN, and Linda Lee, RN assisted with data acquisition and patient coordination.

Corresponding author: Andrew L. Sorenson, MD, 3010 Colby St. #114, Berkeley, CA 94705, USA. Email: alsorenson@gmail.com.

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS
Published by Wolters Kluwer Health, Inc.

0886-3350/$ - see frontmatter
https://doi.org/10.1097/j.jcrs.0000000000000070

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

Table 1. Procedural modifications used to eradicate post-LASIK DLK.

Item	Action	Effect on DLK Incidence
Disposable surgical items		
Surgical gloves	Changed	No effect
Surgical sponge spears	Changed the manufacturer	No effect
Balanced salt solution cannulas	Switched to disposable cannulas	No effect
Goggles	Considered whether adhesive from goggle padding could have effect. Goggles changed to Duo-Shades DS2C-12 light tint	No effect
Microkeratome blade lot numbers	Substituted new lot numbers from time to time, finally switching to disposable heads from reusable heads	No effect
Reusable surgical items		
Microkeratome head	Sent back to Moria multiple times for servicing; switched heads during DLK cluster	No effect
Moria drive motor	Set back to Moria for servicing multiple times	No effect
Flap lifter	Changed to different instrument	No effect
Johnson Applanator	Stopped using	No effect
Speculum	Wire speculum. No changes made	—
Tonometer	No changes made	—
Liquids used during surgery		
Povidone–iodine lid cleanser	No changes made	—
Ink markers and ink wells	Gentian violet ink pads, black ink markers, and no ink all attempted.	No effect
Anesthetics	Tetracaine, Alcaine, and sterile dosed packs used variably	No effect
Steroids	Switched fluorometholone to Pred Forte, Durezol, or no steroid for post-op period.	No effect
Antibiotics	Vigamox, Zymar, Zymaxid, and Ciloxan used variably	No effect
NSAIDs	Acular, Voltaren, and Acuvail used variably	No effect
Balanced saline solution	Alcon BSS Plus, lot numbers routinely cycled over time	No effect
Instrument-cleansing fluids	Switched from Universal Cleaner to Ensol	No effect
Distilled water for reservoir	Changed from Alhambra to Arrowhead	No effect
Modification to technique or other changes		
Extended instrument sterilization drying time	Increased from 35 minutes per tray to 55 minutes per tray	No effect
Intrasurgery flap drying time before administration of drops	Increased from 45 seconds to 2 minutes	No effect
Air filters in the surgical suite	All changed, replaced April 2012. Turned off during the month of May 2012 in the instrument room. Continuous use of high efficiency particulate air (HEPA) purifier initiated March 14, 2012.	No effect
Volume of balanced salt solution used to irrigate the interface before flap repositioning	Volume per eye increased from 0.75 to 3.25 mL.	No effect
Autoclave cassettes, tubing, and filters	Autoclaves serviced by the manufacturer multiple times. Filters, tubing, and cassettes that hold the instruments were all changed.	No effect
Autoclave reservoir	Replaced with the arrival of new autoclaves. Reservoir wall later cultured, revealing *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex	DLK rate returns to baseline

BSS = balanced salt solution; DLK = diffuse lamellar keratitis; LASIK = laser in situ keratomileusis; NSAIDs = nonsteroidal antiinflammatory drug
The noted changes were implemented serially between May 2011 and June 2012 when the rate of DLK was 37.2%. The replacement of the autoclave with its attendant reservoir terminated the cluster of DLK, returning the rate to 2.2%.

perception that endotoxin is the sole bacterial byproduct triggering innate immunity and inflammation is now considered inaccurate. Biofilms harbor a wide variety of other biotoxins that include bacterial and fungal cell wall proteins, bacterial flagellin, DNA and RNA fragments, and microbial exotoxins. All have the potential to produce severe inflammation mediated by the natural system of innate immunity.

We report a cluster of DLK cases most likely caused by biotoxins originating from a well-developed biofilm on the inner wall of a reservoir-based tabletop autoclave (STATIM 2000). We reviewed the literature on DLK and TASS, as well as the contemporary science of innate immunity, to develop and support this hypothesis. Finally, we report a decontamination and cleaning protocol designed to prevent the accumulation of autoclave reservoir biofilm, which has prevented further anomalous DLK at this center.

METHODS

This study was conducted under a protocol approved by the Committee for the Protection of Human Subjects, University of

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

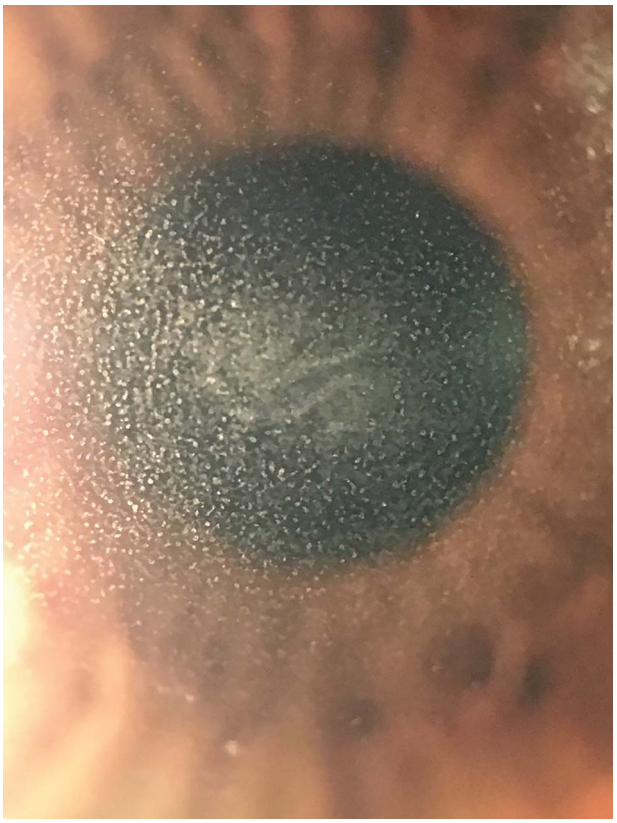

Figure 1. Central toxic keratitis or grade 4+ DLK. Photograph of 1 of 5 eyes with CTK during the cluster of DLK cases. Note the inflammatory cells in the central cornea at the level of the LASIK flap interface. Organizing inflammatory cells centrally can lead to scarring and vision loss. Final corrected distance visual acuity 20/20 after treatment with topical and oral steroids (CTK = central toxic keratopathy; DLK = diffuse lamellar keratitis; LASIK = laser in situ keratomileusis).

California at Berkeley. Between January 2010 and May 2011, patients presenting for refractive surgery to the Refractive Surgery Center at the University of California, Berkeley, School of Optometry, underwent a standardized presurgery workup for refractive surgery. After their refractive surgery, all patients were examined on the first postoperative day by a single observer (K.T.). If any grade of DLK was detected, patients were then examined by the ophthalmic surgeon (A.L.S.). Uncomplicated patients were next examined on day 4 and then at 1 month, 3 months, and 6 months. If DLK was detected, follow-up visits were conducted every 1 to 3 days to monitor response to treatment. Throughout the period during which an elevated incidence of DLK was detected, various modifications in technique were used in an attempt to identify and eliminate the cause (Table 1).

Statistical Analysis

Data are expressed as the ratio of DLK cases relative to the total number of LASIK cases. Chi-squared analysis is used to determine statistical significance of differences between groups. *P* values of less than .05 are considered statistically significant.

Literature Review

The MEDLINE database was searched using the following keywords: TASS, DLK, TASS, DLK, biofilm, and autoclave reservoir contamination. Relevant articles were reviewed to assess the manner in which autoclave reservoirs were analyzed or implicated.

RESULTS

During the 22 months between September 2, 2010, and June 11, 2012, the DLK incidence at the Berkeley center increased from 4 cases in 88 (4.6%) to 147 cases in 395 (37.2%). The clinical course of 7 eyes with grade 4 DLK (central toxic keratopathy) was prolonged and required topical antiinflammatory treatment, intraocular pressure–lowering medications, and, in several cases, oral prednisone (Figure 1). The final 1-year corrected distance visual acuity (CDVA) was reduced to 20/25 in 2 of these 7 eyes. All other eyes recovered a CDVA of 20/20 or 20/25 in the case of 1 amblyopic eye. Four of the 7 eyes required refractive surgical enhancement. In addition, 1 eye developed primary epithelial ingrowth requiring surgical debridement, and 2 eyes developed early cataracts, potentially related to steroid use.

Once the elevated incidence of DLK was recognized, multiple modifications in products, techniques, and instruments were initiated as listed in Table 1. Every modification ultimately failed to halt the excessive DLK rate until the STATIM 2000 (SciCan) autoclave was replaced on June 6, 2012, and a new reservoir sterilization and surveillance was initiated. During the next 31 months, the incidence of DLK dropped to a consistently low baseline rate of 2.2% (14/632 cases), $P < .00001$ (Table 2 and Figure 2).

The retired autoclave was stored with its reservoir drained until the DLK cluster had completely resolved. On April 4, 2013, the dry reservoir chamber sidewalls were cultured for bacterial and fungal contamination, revealing heavy growth of *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex. In the absence of any reservoir moisture, these bacteria presumably colonized the reservoir wall during the use of the autoclave and remained viable despite dry storage for 10 months.

Table 2. DLK incidence between January 1, 2010, and December 18, 2014.

Date	No. LASIK	No. DLK	DLK Rate	Grade 4 DLK	Protocol
January 1, 2010, to June 3, 2010	88	4	4.6%	0	Instructions for use*
September 2, 2010, to June 11, 2012	395	145†	36.7%	5 cases‡	Modified
August 2, 2012, to December 18, 2014	632	14	2.2%	0	Reservoir sterilization

CTK = central toxic keratopathy; DLK = diffuse lamellar keratitis; LASIK = laser in situ keratomileusis

*Instructions for use by SciCan for STATIM 200/5000, Manual 95-108027 Revision 3.0, Copyright 2004. Guidelines for maintaining the autoclave and reservoir were strictly followed.

†Thirty-nine trace DLK (9.9%), 63 grade 1 + DLK (15.9%), 36 grade 2 + DLK (9.1%), 2 grade 3 + DLK (0.5%), and 5 grade 4 + DLK (or CTK, 1.8%).

‡$P < .00001$, χ^2 test, comparing second and third date ranges.

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

Table 3. Possible sources of TASS following cataract surgery, reported between 1992 and 2019.

Author	TASS Cases Reported	Attributed To	Specific Consideration of the Autoclave Reservoir or Biofilm
Richburg et al.[5]	21	Ultrasonic cleaning solution	Not mentioned
Monson et al.[6]	3	Not attributed	Not mentioned
Kreisler et al.[7]	5	Cleaning bath detergent	Not mentioned
Smith et al.[8]	10	Plasma gas protocol	Attributed to AbTox Plazlyte sterilization
Hellinger et al.[9]	8	Steam impurities	Partially attributed to impure steam, potentially from autoclaves
Mamalis[10]	Review	Multiple causes	Autoclaves mentioned, not emphasized
Moshirfar et al.[11]	1	Verisyse lens	Not mentioned
Unal et al.[12]	6	Glutaraldehyde toxicity	Not mentioned
Werner et al.[12]	8	Poor wound construction; ointment contamination of aqueous	Not mentioned
Holland and Morck[13]	14	Endotoxin contamination	Attributed to reservoirs
CDC[14]			—
Holland et al.[15]	Review	Multiple causes	Autoclaves mentioned, not emphasized
Hellinger et al.[16]	White paper	Multiple causes	Not mentioned
Kutty et al.[17]	112	Cytosol	Not mentioned
Sarobe Carricas et al.[18]	5	Uncertain	Not mentioned
Choi and Shyn[19]	15	Sterilization technique	Autoclaves mentioned, not emphasized
Myrna et al.[20]	Letter	Multiple causes in veterinary medicine	Not mentioned
Buzard et al.[21]	2	Trypan blue	Not mentioned
Cutler Peck et al.[22]	Review	Instrument handling	Not mentioned
Jun and Chung[23]	1	Antiseptic solution	Not mentioned
Ozcelik et al.[24]	14	Multifactorial	Not mentioned
Kremer et al.[25]	1	Multivisc BD in phakic IOLs	Not mentioned
Sengupta et al.[26]	60	Multifactorial	Not mentioned
Van Philips[27]	1	Verisyse lens	Not mentioned
Ari et al.[28]	19	Multifactorial and vitrectomy packs	Not mentioned
Bodnar et al.[29]	Review	Instrument baths and multifactorial	Not mentioned*
Moisseiev and Barak[30]	4	Silicone oil	Not mentioned
Moyle et al.[31]	11+	Undetermined, list of 34 in DDX	Autoclaves mentioned, not emphasized
Kumaran et al.[32]	1	Hoya Surgical Optics IOL	Not mentioned
Cetinkaya et al.[33]	5	Undetermined	Not mentioned
Althomali[34]	15	Viscosurgical	Not mentioned
Sorenson et al.[35]	10	Autoclave reservoir biofilm	Attributed to reservoirs
Altintas et al.[36]	34	Viscosurgical	Not mentioned
Matsou et al.[37]	5	Generic trypan blue	Not mentioned
Oshika et al.[38]	147†	Specific model acrylic IOL	Not mentioned
Park et al.[39]	Review	Multiple causes	Autoclaves mentioned, not emphasized
Singh et al.[40]	1	Implantable collamer lens	Not mentioned
Farooqui et al.[41]	81	Endotoxin from viscosurgical device	Not mentioned
Hernandez-Bogantes et al.[42]	6	Powdered gloves	Not mentioned
Hernandez-Bogantes et al.[43]	Survey	Multifactorial	Autoclaves mentioned

IOL = intraocular lens; TASS = toxic anterior segment syndrome
Several authors suspect the autoclave, but many studies, including larger reviews, do not mention the possible role of the autoclave reservoir with its potential for biofilm contamination.
*Authors specifically mention inadequate adherence to the manufacturer's IFU at that time.
†Subacute onset likely decategorizes this patient population as a TASS population.

Literature Review

Forty case reports, reviews, and other articles in the peer-reviewed literature between 1986 and 2019 relating to the topic of TASS[5–44] (Table 3). Another 22 reports and articles on DLK[1–3,45–63] (Table 4) were identified. Only 5 of these 62 total studies reported specifically investigating the potential role of autoclave reservoirs and their associated biofilms as the potential cause of TASS or DLK.[3,13,35,46,52]

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

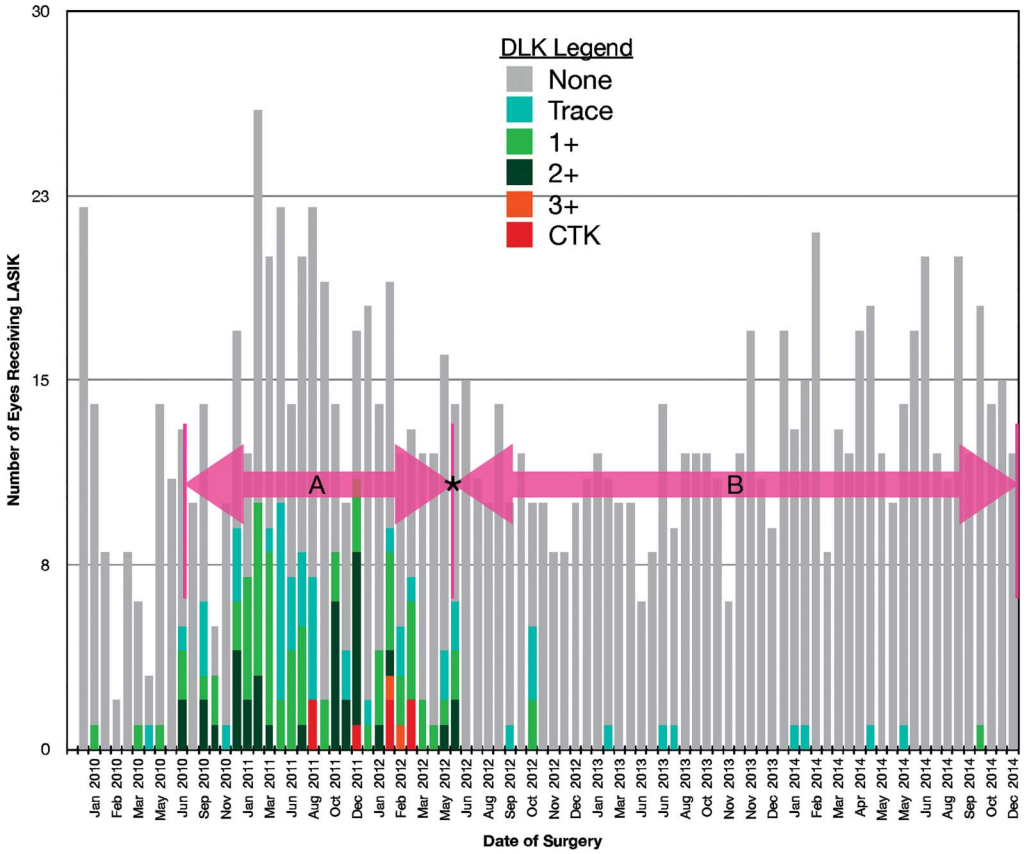

Figure 2. Timeline of LASIK cases and DLK, 2010 to 2014. Incidence of DLK after LASIK at a single surgery center over a period of 5 years. DLK cases are color-coded to indicate severity of inflammation and plotted in relation to total LASIK cases. DLK cluster (timespan A) concludes after the autoclave is replaced at the asterisk. During timespan B, the new autoclave reservoir is sterilized before each OR date, and the reservoir wall is cultured each month for 12 months returning a report of "no growth" on each occasion. Abnormal incidence of DLK terminated with the new autoclave and reservoir sterilization protocol. Instruments used on the first OR date after the initiation of the new autoclave had been previously sterilized in the old autoclave, allowing these cases to be attributed to the previous autoclave. *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex were cultured from the retired autoclave reservoir wall (DLK = diffuse lamellar keratitis; LASIK = laser in situ keratomileusis; OR = operating room).

DISCUSSION

Biofilms are polymicrobial cell populations that attach themselves to moist surfaces in a prolonged and durable manner and persist once the surface is dry. With sufficient time, virtually all moist surfaces are likely to develop a biofilm potentially comprising Gram-negative and positive bacterial and fungal elements. As the biofilm develops, it encloses itself in a matrix and becomes resistant to simple removal such as by rinsing with sterile water.[64,65] Highly concentrated antibiotics, physical scrubbing, or exposure to boiling water may be necessary to remove the biofilm.[66,67]

Clinically, exposure to the constituents of biofilms can elicit severe immune responses resulting in damage to host tissues. Because of its small size and anterior chamber volume, the eye is especially sensitive. These immune responses involve the innate immune system generating a series of antimicrobial and inflammatory defenses in response to common microbial antigens. In contrast to acquired immunity, innate immune responses are considered to be nonspecific and lack immunological memory, that is, they target a broad range of microbes and are not boosted by previous exposure to antigen.

The scientific understanding of the system of innate immunity has expanded dramatically since 2000, when sterilizer reservoir biofilms were first implicated by Holland et al. in a cluster of 52 cases of DLK.[50] Subsequently, the central role of PRRs in innate immunity was established. PRRs are present on and within inflammatory cells of many species where they detect the presence of pathogens by recognizing molecules unique to microorganisms. Initially thought to primarily recognize endotoxin, PRRs also mediate inflammatory responses to other bacterial and fungal cell wall proteins, bacterial flagellin, DNA and RNA fragments, and other microbial exotoxins. All of these byproducts of microbial cellular damage might be generated when microorganisms are exposed to high autoclave temperatures or steam.

If biological contaminants of a reservoir wall biofilm enter the autoclave chamber, they may be inoculated onto the surface of exposed ophthalmic instruments. The bacteria and fungi will be killed, and other byproducts will be broken down by heat and steam. However, fragments of these inactivated microbial byproducts may persist whereupon they could be introduced into the anterior chamber or corneal stroma in sufficient amounts to trigger PRR-mediated TASS or DLK.

We report a prolonged DLK cluster that continued despite multiple systematic changes in instrumentation and surgical protocols. This cluster of cases eventually ended following replacement of the entire tabletop autoclave. The absence of any further spikes in the DLK rate implicated the autoclave as the cause of our cluster. Therefore, the inner reservoir wall of the original autoclave was swabbed and cultured 10 months after it had been

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

Table 4. Possible sources of diffuse lamellar keratitis following LASIK, reported between 2000 and 2019.

Author	DLK Cases (%)	Attributed To	Autoclave Reservoir
Smith and Maloney[45]	13	Allergic or toxic; non-infectious	Not mentioned
Holland[46]	52/928 (5.3)	Reservoir biofilm, conversion to endotoxin, and *Burkholderia pickettii*	Attributed to autoclaves
Linebarger[47]	1/25 to 1/5000 (≤4)	Not attributed	Not mentioned
Fogla[48]	Commentary	Meibomian gland secretions	Not mentioned
Johnson et al.[2]	36/2711 (1.3)	Epithelial defects, blood, and trauma of surgery,	Not mentioned
Yuhan[49]	15/210 (7.1)	Stagnant fluids used in cleaning instruments	Autoclaves mentioned, not emphasized
Wilson[50]	17/1352 (1.3)	Host factors, eg, IL-1	Autoclaves mentioned, not emphasized
Ambrósio[51]	Case report of 4 eyes	Meibomian gland disease	Not mentioned
Stulting et al.[3]	54/11,232 (0.5)	Primary LASIK vs enhancements	Attributed to autoclaves
Villarrubia[52]	46/188 (24.5)	Bacterial endotoxin, *B pickettii*, and *Sphingomonas paucimobilis*	Attributed to autoclaves
Moshirfar[53]	193/10477 (1.8)	Epithelial defects or idiopathic	Autoclaves mentioned, not emphasized
Gil-Cazorla et al.[1]	6/2000 (0.3)	Steroid dosing	Not mentioned
Choe[54]	64/520 (12.3)	Attributed to femtosecond lasers	Not mentioned
Moshirfar[55]	150/1798 (8.3)	Considered normal rate, with higher rate noted in femtosecond created flaps	Not mentioned
Javaloy[56]	300/1161 (26)	Sex, atopy, drug allergies, treatment magnitude, and microkeratome use.	Partially attributed to autoclaves*
Gritz[57]	Review	Patient variation (atopia, ocular disease, blepharitis, and epithelial defects)	Autoclaves mentioned, not emphasized
de paula[58]	99/801 (12.4)	Femtosecond raster energy settings, flap diameter, and side cut	Not mentioned
Randleman[59]	Review	Autoclave reservoirs implicated in some cases	Autoclaves emphasized
Tomita et al.[60]	156	Not determined	Not mentioned
Kymionis et al.[61]	1	Corneal blood vessels	Not mentioned
Abdelmaksoud et al.[62]	2 (CTK)	Not determined	Not mentioned
Balestrazzi et al.[63]	1	Not attributed	Not mentioned
Sorenson (this paper)	147/395	Reservoir contamination	Attributed to autoclaves

DLK = diffuse lamellar keratitis; CTK = central toxic keratopathy; LASIK = laser in situ keratomileusis
*Statim autoclave noted to be the autoclave in use and author suggests autoclave may be related to the DLK outbreak. Several authors suspect the autoclave, but many studies, including larger reviews, do not mention the possible role of the autoclave reservoir.

drained and retired. Despite regular draining and drying of the autoclave reservoir while in use, a polymicrobial population consistent with a well-developed biofilm was cultured from its walls. Both *P aeruginosa* and *B cepacia* bacteria cultured from the reservoir wall produce known ligands (eg, LPS, flagellin) to which PRRs can respond. We believe that these small molecular biotoxins, derived from the autoclave reservoir wall biofilm, were seeded onto the surgical instruments and then introduced into the cornea stroma. DLK was the innate immune response mediated by PRRs. Moreover, our ability to culture these microbes after an extended period of dry storage reflects the tenacity with which bacterial subpopulations are able to survive in a dormant state within a biofilm.[68,69]

Table 5. Cleaning STATIM 900, 2000, and 5000 autoclave reservoirs in ophthalmic surgery settings.

For cleaning recommendations of the cassette and cabinet in our STATIM Cassette Autoclave, see the operator's manual. Although the reservoir is not a sterile area, the steam-processed distilled water used in the reservoir is rapidly converted to steam and itself becomes sterilized in the autoclave. To ensure a clean reservoir, we recommend that the following preliminary procedure be performed weekly:

Step 1. Turn the unit's power switch to the "OFF" position.
Step 2. Remove the reservoir cap and, if present, the coarse mesh filter.
Step 3. Completely drain the reservoir by means of the drain tube.
Step 4. Completely fill the reservoir (≈4.0 L) with boiling distilled water (do not use tap water).
Step 5. Allow boiling water to remain in the reservoir for 2 to 3 min.
Step 6. Drain the autoclave reservoir completely (using the drain tube) until next use.
Step 7. Before next use, fill the reservoir with distilled water (room temperature).
Step 8. Turn the power switch to the "ON" position, use as per the operator's manual.

Guidelines developed in cooperation with SciCan.

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

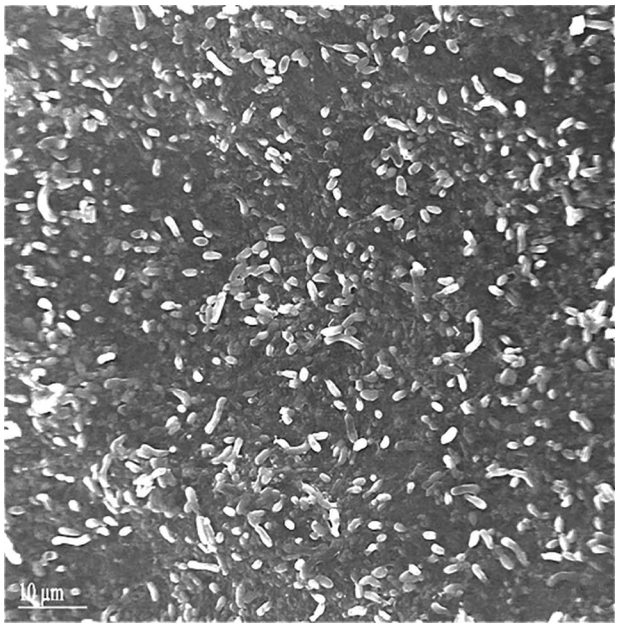

Figure 3. SEM of the autoclave wall section removed from the TASS-implicated autoclave reservoir Reprinted with permission from the the *Journal of Cataract & Refractive Surgery*.[35] SEM = scanning electron microscopy; TASS = toxic anterior segment syndrome.

A 2016 review of autoclave reservoir biofilms by Sorenson et al. surveyed regional outpatient surgery center tabletop autoclaves in association with a single-center cluster of TASS cases.[35] Scanning electron micrographs were taken of a section of the autoclave reservoir wall obtained from the retired autoclave used to sterilize instruments implicated in 10 cases of TASS. These showed a well-developed biofilm where many bacterial elements appear to be in the predispersal phase (Figure 3). Calculations of reservoir wall bacterial density from these images suggest a surface population of approximately 10 billion cells, and a total population of many times that figure, given the multilayered nature of biofilms. Conceivably, their byproducts and dispersal elements could contaminate the fluid drawn into the reservoir to produce steam. In that

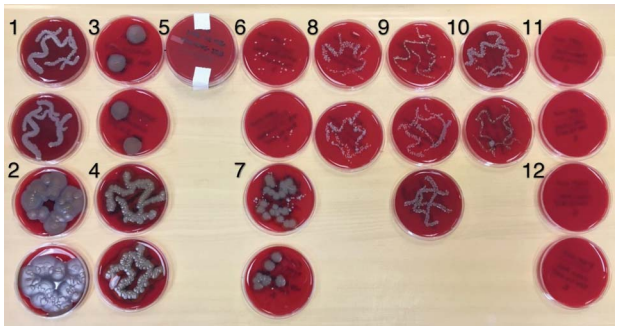

Figure 4. Blood agar plates inoculated with samples from 12 regional ASC autoclaves. All samples are taken from the surface of the STATIM tabletop autoclave reservoir inner wall. After 48 hours of incubation, 10 of the 12 reservoirs demonstrated culturable biofilm, as shown.[35]

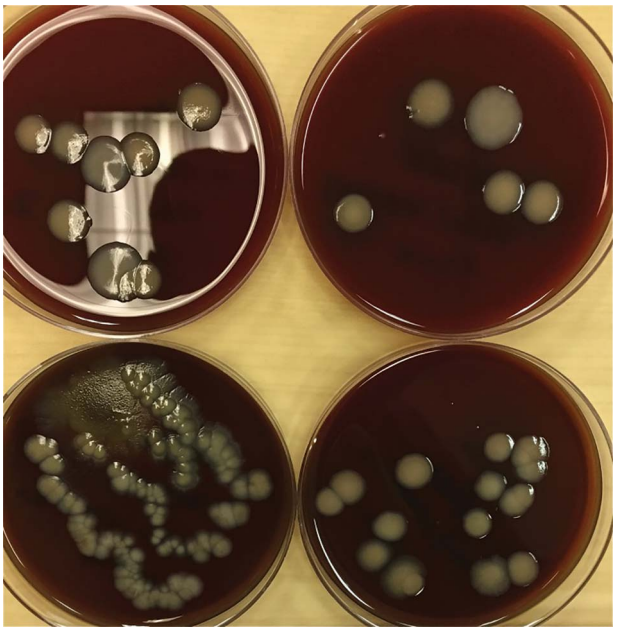

Figure 5. Culture of a new STATIM autoclave reservoir from a different surgery center after 20 days on initial use. The reservoir wall from a STATIM autoclave purchased as part of another study and not yet exposed to the boiling water protocol, was cultured after 20 days of use. These plates were incubated for 7 days and demonstrated contamination.[35]

study, 20 of the 25 autoclave reservoirs surveyed from regional ambulatory surgery centers demonstrated biofilm contamination, and 19 different bacterial or fungal species were identified.[35] Figure 4 shows the blood agar plates of 12 such autoclaves.

Between 1986 and 2019, 40 articles on TASS were published in the peer-reviewed literature. Another 22 papers were published on DLK. Most of these preceded the description of PRRs and their role in innate immunity,

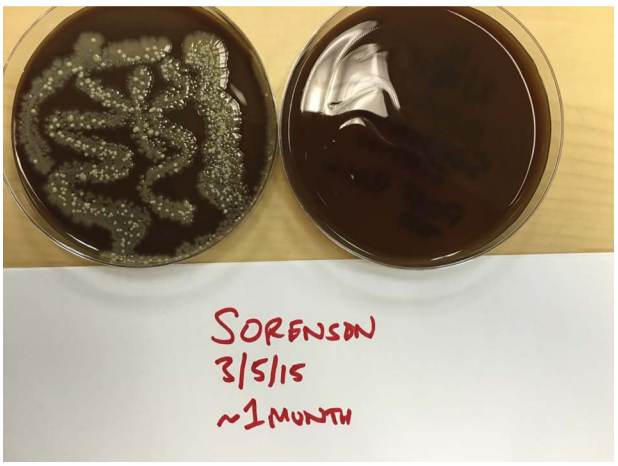

Figure 6. Biofilm not culturable after exposure to boiling water. Left: plate from the inner wall swabbing of a STATIM autoclave reservoir inner wall before the initiation of the boiling water cleaning protocol. Right: plate inoculated after exposure of the reservoir to boiling water for 2 to 3 minutes. After 1 month, still no growth of bacteria or fungi.

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

which now implicates a more expansive list of microbial byproducts besides heat-stable endotoxin. This may have led the authors to overlook or underestimate the potential for inactivated biofilm components from the autoclave reservoir to cause TASS and DLK. For example, only 5 of these 62 articles report investigating autoclave reservoirs as a potential etiology (Tables 3 and 4). As a result, we believe that the ophthalmic surgical community remains largely unaware of the potential risks of biofilm formation and accumulation on the reservoir walls of steam autoclaves.

To reduce biofilm formation, the autoclave reservoir should be regularly drained and air dried, such as at the conclusion of each week's usage. However, this alone may not prevent biofilm formation. For example, a newly purchased autoclave reservoir developed culturable biofilm after only 20 days of use despite being drained and dried after each day of surgery (Figure 5). This finding was consistent with Holland's 1999 study demonstrating biofilm contamination of glass beads after 11 days of exposure to distilled water within a previously decontaminated autoclave reservoir. If the autoclave reservoir design precludes scrubbing or physical removal of biofilm, thermal destruction with boiling water is probably the best method to prevent and remove biofilm formation from the heat-stable plastic wall. Indeed, exposure to boiling water effectively eliminated biofilm viability in all 3 treated reservoirs from our 2016 data (Figure 6 shows one such culture set). Accordingly, we adopted this boiling water cleaning protocol for the reservoir of the STATIM autoclave, which replaced the older unit at the Berkeley ASC. Monthly cultures of this autoclave reservoir were negative for 12 consecutive months from August 2012 to August 2013, and similar surveillance cultures might be considered by other centers who adopt this protocol to establish its utility. We have continued the following maintenance protocol at the Berkeley center on a weekly basis since the resolution of the DLK cluster (Table 5):

Step 1. Turn the unit's power switch to the "OFF" position.
Step 2. Remove the reservoir cap and, if present, the course mesh filter.
Step 3. Completely drain the reservoir by means of the drain tube.
Step 4. Completely fill the reservoir (≈4.0 L) with boiling distilled water (do not use tap water).
Step 5. Allow boiling water to remain in the reservoir for 2 to 3 minutes.
Step 6. Drain the autoclave reservoir completely (using the drain tube) until next use.
Step 7. Before next use, fill the reservoir with distilled water (room temperature).
Step 8. Turn the power switch to the "ON" position, use as per the operator's manual.

Note that the inserted cassette required for the cycle contains no instruments, and that the near-capacity filling allows boiling water to contact the dependent and vertical surfaces of the autoclave reservoir wall.

We concluded that inactivated contaminants from the autoclave reservoir biofilm caused the cluster of DLK at the Berkeley center. Since the initiation of this cleaning protocol, the incidence of DLK has remained below 2.2% at this same center. Although this is an anecdotal observation from a single center, when considered together with 2 previously published clusters caused by reservoir contamination, we believe that reservoir wall biofilms are occult sources of steam contamination and pose an underappreciated risk for triggering DLK or TASS.[35,50] After consulting with and reviewing this protocol with the manufacturer (SciCan), we endorse regular reservoir cleansing with boiling water in STATIM cassette autoclaves in an effort to mitigate this risk.

WHAT WAS KNOWN

- Toxic anterior segment syndrome (TASS) and diffuse lamellar keratitis (DLK) are uncommon inflammatory outcomes after uncomplicated ocular surgery. Many suspected causes have been suggested.
- Biofilms are endemic to nearly all moist surfaces and undergo a transformation rendering their microenvironment resistant to simple elimination.
- Biofilms harbor a wide variety of other biotoxins that include bacterial and fungal cell wall proteins, flagellin, DNA and RNA fragments, and microbial exotoxins, all of which stimulate the system of innate immunity via pattern recognition receptors (PRRs).

WHAT THIS PAPER ADDS

- The literature discussing TASS and DLK may have overlooked the role of autoclave reservoir biofilms because the understanding of PRRs and their role in innate immunity has only come to light in recent years.
- Fluid reservoirs of tabletop steam autoclaves can readily develop polymicrobial biofilms harboring microbial pathogens, whose inert molecular byproducts can cause DLK and TASS when introduced to the eye by surgical instruments.
- Stringent reservoir cleaning and maintenance may significantly reduce this risk by preventing and removing these biofilms. Ophthalmic surgical centers should consider following the reservoir sterilization protocol presented herein.

REFERENCES

1. Gil-Cazorla R, Teus MA, de Benito-Llopis L, Fuentes I. Incidence of diffuse lamellar keratitis after laser in situ keratomileusis associated with the IntraLase 15 kHz femtosecond laser and Moria M2 microkeratome. J Cataract Refract Surg 2008;34:28–31
2. Johnson JD, Harissi-Dagher M, Pineda R, Yoo S, Azar DT. Diffuse lamellar keratitis: incidence, associations, outcomes, and a new classification system. J Cataract Refract Surg 2001;27:1560–1566
3. Stulting RD, Randleman JB, Couser JM, Thompson KP. The epidemiology of diffuse lamellar keratitis. Cornea 2004;23:680–688
4. Bühren J, Baumeister M, Cichocki M, Kohnen T. Confocal microscopic characteristics of stage 1 to 4 diffuse lamellar keratitis after laser in situ keratomileusis. J Cataract Refract Surg 2002;28:1390–1399
5. Richburg FA, Reidy JJ, Apple DJ, Olson RJ. Sterile hypopyon secondary to ultrasonic cleaning solution. J Cataract Refract Surg 1986;12:248–251
6. Monson MC, Mamalis N, Olson RJ. Toxic anterior segment inflammation following cataract surgery. J Cataract Refract Surg 1992;18:184–189
7. Kreisler KR, Martin SS, Young CW, Anderson CW, Mamalis N. Postoperative inflammation following cataract extraction caused by bacterial contamination of the cleaning bath detergent. J Cataract Refract Surg 1992;18:106–110

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

8. Smith CA, Khoury JM, Shields SM, Roper GJ, Duffy RE, Edelhauser HF, Lubniewski AJ. Unexpected corneal endothelial cell decompensation after intraocular surgery with instruments sterilized by plasma gas. Ophthalmology 2000;107:1561–1566; discussion 1567
9. Hellinger WC, Hasan SA, Bacalis LP, Thornblom DM, Beckmann SC, Blackmore C, Forster TS, Tirey JF, Ross MJ, Nilson CD, Mamalis N, Crook JE, Bendel RE, Shetty R, Stewart MW, Bolling JP, Edelhauser HF. Outbreak of toxic anterior segment syndrome following cataract surgery associated with impurities in autoclave steam moisture. Infect Control Hosp Epidemiol 2006;27:294–298
10. Mamalis N. Toxic anterior segment syndrome. J Cataract Refract Surg 2006;32:181–182
11. Moshirfar M, Whitehead G, Beutler BC, Mamalis N. Toxic anterior segment syndrome after verisyse iris-supported phakic intraocular lens implantation. J Cataract Refract Surg 2006;32:1233–1237
12. Unal M, Yücel I, Akar Y, Oner A, Altin M. Outbreak of toxic anterior segment syndrome associated with glutaraldehyde after cataract surgery. J Cataract Refract Surg 2006;32:1696–1701
13. Werner L, Sher JH, Taylor JR, Mamalis N, Nash WA, Csordas JE, Green G, Maziarz EP, Liu XM. Toxic anterior segment syndrome and possible association with ointment in the anterior chamber following cataract surgery. J Cataract Refract Surg 2006;32:227–235
14. Holland S, Morck D. Autoclave contamination and TASS. J Cataract Refract Surg 2006;2006:58–60
15. Centers for Disease Control and Prevention (CDC). Toxic anterior segment syndrome after cataract surgery—Maine, 2006. MMWR Morb Mortal Wkly Rep 2007;56:629–630
16. Holland SP, Morck DW, Lee TL. Update on toxic anterior segment syndrome. Curr Opin Ophthalmol 2007;18:4–8
17. Hellinger WC, Bacalis LP, Edelhauser HF, Mamalis N, Milstein B, Masket S; ASCRS Ad Hoc Task Force on Cleaning and Sterilization of Intraocular Instruments. Recommended practices for cleaning and sterilizing intraocular surgical instruments. J Cataract Refract Surg 2007;33:1095–1100
18. Kutty PK, Forster TS, Wood-Koob C, Thayer N, Nelson RB, Berke SJ, Pontacolone L, Beardsley TL, Edelhauser HF, Arduino MJ, Mamalis N, Srinivasan A. Multistate outbreak of toxic anterior segment syndrome, 2005. J Cataract Refract Surg 2008;34:585–590
19. Sarobe Carricas M, Segrelles Bellmunt G, Jiménez Lasanta L, Iruin Sanz A. Toxic anterior segment syndrome (TASS): studying an outbreak [in Spanish]. Farm Hosp Órgano Expr Científica Soc Esp Farm Hosp 2008;32:339–343
20. Choi JS, Shyn KH. Development of toxic anterior segment syndrome immediately after uneventful phaco surgery. Korean J Ophthalmol KJO 2008;22:220–227
21. Myrna KE, Pot S, Bentley E, Adkins E, Miller P, Murphy C. Toxic anterior segment syndrome and are we missing it? Vet Ophthalmol 2009;12:138
22. Buzard K, Zhang JR, Thumann G, Stripecke R, Sunalp M. Two cases of toxic anterior segment syndrome from generic trypan blue. J Cataract Refract Surg 2010;36:2195–2199
23. Cutler Peck CM, Brubaker J, Clouser S, Danford C, Edelhauser HE, Mamalis N. Toxic anterior segment syndrome: common causes. J Cataract Refract Surg 2010;36:1073–1080
24. Jun EJ, Chung SK. Toxic anterior segment syndrome after cataract surgery. J Cataract Refract Surg 2010;36:344–346
25. Ozcelik ND, Eltutar K, Bilgin B. Toxic anterior segment syndrome after uncomplicated cataract surgery. Eur J Ophthalmol 2010;20:106–114
26. Kremer I, Levinger E, Levinger S. Toxic anterior segment syndrome following iris-supported phakic IOL implantation with viscoelastic Multivisc BD. Eur J Ophthalmol 2010;20:451–453
27. Sengupta S, Chang DF, Gandhi R, Kenia H, Venkatesh R. Incidence and long-term outcomes of toxic anterior segment syndrome at Aravind Eye Hospital. J Cataract Refract Surg 2011;37:1673–1678
28. van Philips LAM. Toxic anterior segment syndrome after foldable artiflex iris-fixated phakic intraocular lens implantation. J Ophthalmol 2011;2011:982410
29. Ari S, Caca I, Sahin A, Cingü AK. Toxic anterior segment syndrome subsequent to pediatric cataract surgery. Cutan Ocul Toxicol 2012;31:53–57
30. Bodnar Z, Clouser S, Mamalis N. Toxic anterior segment syndrome: update on the most common causes. J Cataract Refract Surg 2012;38:1902–1910
31. Moisseiev E, Barak A. Toxic anterior segment syndrome outbreak after vitrectomy and silicone oil injection. Eur J Ophthalmol 2012;22:803–807
32. Moyle W, Yee RD, Burns JK, Biggins T. Two consecutive clusters of toxic anterior segment syndrome. Optom Vis Sci 2013;90:e11–23
33. Kumaran N, Larkin G, Hollick EJ. Sterile postoperative endophthalmitis following HOYA IOL insertion. Eye 2014;28:1382
34. Cetinkaya S, Dadaci Z, Aksoy H, Acir NO, Yener HI, Kadioglu E. Toxic anterior-segment syndrome (TASS). Clin Ophthalmol 2014;8:2065–2069
35. Althomali TA. Viscoelastic substance in prefilled syringe as an etiology of toxic anterior segment syndrome. Cutan Ocul Toxicol 2016;35:237–241
36. Sorenson AL, Sorenson RL, Evans DJ. Toxic anterior segment syndrome caused by autoclave reservoir wall biofilms and their residual toxins. J Cataract Refract Surg 2016;42:1602–1614
37. Altintas AK, Ciritoglu MY, BeyazyildiZ O, Can CU, Polat S. Toxic anterior segment syndrome outbreak after cataract surgery triggered by viscoelastic substance. Middle East Afr J Ophthalmol 2017;24:43–47
38. Matsou A, Tzamalis A, Chalvatzis N, Mataftsi A, Tsinopoulos I, Brazitikos P. Generic trypan blue as possible cause of a cluster of toxic anterior segment syndrome cases after uneventful cataract surgery. J Cataract Refract Surg 2017;43:848–852
39. Oshika T, Eguchi S, Goto H, Ohashi Y. Outbreak of subacute-onset toxic anterior segment syndrome associated with single-piece acrylic intraocular lenses. Ophthalmology 2017;124:519–523
40. Park CY, Lee JK, Chuck RS. Toxic anterior segment syndrome-an updated review. BMC Ophthalmol 2018;18:276
41. Singh A, Gupta N, Kumar V, Tandon R. Toxic anterior segment syndrome following phakic posterior chamber IOL: a rarity. BMJ Case Rep 2018;11. doi:10.1136/bcr-2018-225806
42. Farooqui JH, Gandhi A, Mathur U, Bharti G, Dubey S. Increased postoperative anterior chamber inflammation secondary to heat-resistant endotoxins. J Cataract Refract Surg 2019;45:188–195
43. Hernandez-Bogantes E, Ramirez-Miranda A, Olivo-Payne A, Abdala-Figuerola A, Navas A, Graue-Hernandez EO. Toxic anterior segment syndrome after implantation of phakic implantable collamer lens. Int J Ophthalmol 2019;12:175–177
44. Hernandez-Bogantes E, Navas A, Naranjo A, Amescua G, Graue-Hernandez EO, Flynn HW Jr, Ahmed I. Toxic anterior segment syndrome: a review. Surv Ophthalmol 2019;64:463–476
45. Smith RJ, Maloney RK. Diffuse lamellar keratitis. A new syndrome in lamellar refractive surgery. Ophthalmology 1998;105:1721–1726
46. Holland SP, Mathias RG, Morck DW, Chiu J, Slade SG. Diffuse lamellar keratitis related to endotoxins released from sterilizer reservoir biofilms. Ophthalmology 2000;107:1227–1233; discussion 1233-1234
47. Linebarger EJ, Hardten DR, Lindstrom RL. Diffuse lamellar keratitis: diagnosis and management. J Cataract Refract Surg 2000;26:1072–1077
48. Fogla R, Rao SK, Padmanabhan P. Diffuse lamellar keratitis: are meibomian secretions responsible? J Cataract Refract Surg 2001;27:493–495
49. Yuhan KR, Nguyen L, Wachler BSB. Role of instrument cleaning and maintenance in the development of diffuse lamellar keratitis. Ophthalmology 2002;109:400–403; discussion 403-404
50. Wilson SE, Ambrósio R. Sporadic diffuse lamellar keratitis (DLK) after LASIK. Cornea 2002;21:560–563
51. Ambrósio R, Periman LM, Netto MV, Wilson SE. Bilateral marginal sterile infiltrates and diffuse lamellar keratitis after laser in situ keratomileusis. J Refract Surg 2003;19:154–158
52. Villarrubia A, Palacín E, Gómez del Río M, Martínez P. Description, etiology, and prevention of an outbreak of diffuse lamellar keratitis after LASIK. J Refract Surg 2007;23:482–486
53. Moshirfar M, Welling JD, Feiz V, Holz H, Clinch TE. Infectious and noninfectious keratitis after laser in situ keratomileusis occurrence, management, and visual outcomes. J Cataract Refract Surg 2007;33:474–483
54. Choe CH, Guss C, Musch DC, Niziol LM, Shtein RM. Incidence of diffuse lamellar keratitis after LASIK with 15 KHz, 30 KHz, and 60 KHz femtosecond laser flap creation. J Cataract Refract Surg 2010;36:1912–1918
55. Moshirfar M, Gardiner JP, Schliesser JA, Espandar L, Feiz V, Mifflin MD, Chang JC. Laser in situ keratomileusis flap complications using mechanical microkeratome versus femtosecond laser: retrospective comparison. J Cataract Refract Surg 2010;36:1925–1933
56. Javaloy J, Alió JL, Rodríguez A, González A, Pérez-Santonja JJ. Epidemiological analysis of an outbreak of diffuse lamellar keratitis. J Refract Surg 2011;27:796–803
57. Gritz DC. LASIK interface keratitis: epidemiology, diagnosis and care. Curr Opin Ophthalmol 2011;22:251–255
58. de Paula FH, Khairallah CG, Niziol LM, Musch DC, Shtein RM. Diffuse lamellar keratitis after laser in situ keratomileusis with femtosecond laser flap creation. J Cataract Refract Surg 2012;38:1014–1019
59. Randleman JB, Shah RD. LASIK interface complications: etiology, management, & outcomes. J Refract Surg 2012;28:575–586
60. Tomita M, Sotoyama Y, Yukawa S, Nakamura T. Comparison of DLK incidence after laser in situ keratomileusis associated with two femtosecond

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.

lasers: femto LDV and IntraLase FS60. Clin Ophthalmol Auckl NZ 2013;7:1365–1371
61. Kymionis GD, Tsoulnaras KI, Tsakalis NG, Grentzelos MA. Diffuse lamellar keratitis in the femtosecond-assisted LASIK flap tunnel. Clin Ophthalmol Auckl NZ 2014;8:1065–1067
62. Abdelmaksoud A, Khoo NT, Hanoot H, Ibrahim O. Bilateral central toxic keratopathy after laser in situ keratomileusis. BMJ Case Rep 2015;2015;bcr2015212423
63. Balestrazzi A, Balestrazzi A, Giannico MI, Michieletto P, Balestrazzi E. Diagnosis, clinical trend, and treatment of diffuse lamellar keratitis after femtosecond laser-assisted in situ keratomileusis: a case report. Case Rep Ophthalmol 2018;9:457–464
64. Teschler JK, Zamorano-Sánchez D, Utada AS, Warner CJ, Wong GC, Linington RG, Yildiz FH. Living in the matrix: assembly and control of Vibrio cholerae biofilms. Nat Rev Microbiol 2015;13:255–268
65. Maunders E, Welch M. Matrix exopolysaccharides; the sticky side of biofilm formation. FEMS Microbiol Lett 2017;364. doi:10.1093/femsle/fnx120
66. World Health Organization. Boil Water Technical Brief. 2015. Available at: https://www.who.int/water_sanitation_health/publications/boiling-water/en/. Accessed June 21, 2019
67. Sharahi JY, Azimi T, Shariati A, Safari H, Tehrani MK, Hashemi A. Advanced strategies for combating bacterial biofilms. J Cell Physiol [Epub ahead of print January 29, 2019.]
68. Akiyama T, Williamson KS, Schaefer R, Pratt S, Chang CB, Franklin MJ. Resuscitation of Pseudomonas aeruginosa from dormancy requires hibernation promoting factor (PA4463) for ribosome preservation. Proc Natl Acad Sci U S A 2017;114:3204–3209
69. Tam C, Mun JJ, Evans DJ, Fleiszig SMJ. The impact of inoculation parameters on the pathogenesis of contact lens-related infectious keratitis. Invest Ophthalmol Vis Sci 2010;51:3100–3106

Disclosures: *None of the authors has a financial or proprietary interest in any material or method mentioned.*

Copyright © 2020 Published by Wolters Kluwer on behalf of ASCRS and ESCRS. Unauthorized reproduction of this article is prohibited.