The 2015 Don Korb Award Lecture: From Corneal Infiltration to Transplantation

Loretta Szczotka-Flynn OD, PhD, FAAO
AAO Diplomate; Section on Cornea and Contact Lenses
Professor
Case Western Reserve University Department of Ophthalmology
Director: Contact Lens Service
University Hospitals Case Medical Center Eye Institute
Director: Coordinating Center
Cornea Preservation Time Study

Disclosures in the Past 12 months: Menicon, Alcon Research Ltd, Johnson & Johnson Vision Care

Contact Lens associated Inflammatory Events (CIEs)
Recurrent Contact Lens associated Inflammatory Events (CIEs)

WHAT'S THE RELATIVE RISK?
The Silicone Hydrogel Effect

Confounding
- Almost all SH studies used SH lenses worn for 30 days CW
- Almost all low Dk studies used lenses worn for 7 days EW
Other studies documenting a silicone hydrogel effect during DW or EW

Radford et al UK Case Control Study 2009
- 877 Cases with non-ulcerative complications
- 1069 hospital and 639 population controls
- Si-Hy increased risk for sterile keratitis
  - Independent from mode of wear
    - 2.0 X
- Si-Hy also associated with
  - Mechanical disorders
    - 1.8X
  - Attendance with any non-ulcerative complication
    - 1.9X

Other studies documenting a silicone hydrogel effect on CIEs

Chalmers et al OVS 2010
- 1276 soft lens wearers, retrospective chart review
- Silicone hydrogel lenses increased risk of inflammatory events
  - Hydrogel lenses were protective (0.77 RR)
  - Controlled for mode of wear

Chalmers et al IOVS 2011
- CLAY Study
- 3549 soft lens wearers, retrospective chart review
  - 187 CIEs in 168 patients
- Silicone hydrogel lenses increased risk of inflammatory events
  - 1.85X
  - Controlled for mode of wear
Other studies documenting a silicone hydrogel effect on CIEs

- Chalmers et al OVS 2012
  - Case Control Study
  - 166 patients with symptomatic CIEs
  - Silicone hydrogel increased risk of CIE
    - 1.99X Daily Wear
      - Extended wear did not find SH to increase risk

Survival Curve of remaining CIE free during EXTENDED WEAR with a Silicone Hydrogel Material

EFFECT OF LENS BIOBURDEN

Univariate Hazard Ratio through 12 months
4.41 (95% CI 2.21-8.79)

What is the incidence of CIEs during daily wear?

- 3-20%
  - ~3% presenting for care
  - Two large, multicenter, retrospective chart reviews, optometry school clinics, 6,117 patient-years of contact lens wear (Chalmers et al, 2011; Chalmers et al, 2011).
  - ~7% all CIEs, 2% symptomatic
  - Single center, prospective, lotrafilcon A (Szczotka-Flynn 2014)
  - ~20% (annualized to 1 year)
  - university-based, series of 3-month, nonrandomized, prospective studies, n=368, variety of silicone hydrogel lenses for daily wear with modern lens care products (Carnt et al, 2009).
The TEMPO Registry Cohort

- Prospective registry of patients being fit with Daily Disposable CLs (not randomized):
  - 1•DAY ACUVUE® TruEye®
  - 1•DAY ACUVUE® MOIST®
- Enrolled October 15, 2011 to August 31, 2012
- 38 Sites, 83 Clinicians
- 953 habitual SCL wearers with known brands
- ≥ 20 SCL brands & ≥ 12 lens care brands

Chalmers, et al, Johnson & Johnson Vision Care ISCLR 2013

SCL-Related Problems in the TEMPO Registry Cohort

- Corneal Infiltrates in 3.0%
- Only 0.3% were solution hypersensitivity

Chalmers, et al, Johnson & Johnson Vision Care ISCLR 2013

Influence of Lens Care Systems on Lens Materials and CIEs

Effect of Lens Care Systems on the Clinical Performance of a Contact Lens

Table 3: Estimated adjusted mean and 95% confidence interval for contact on insertion and contact post-dose; change at the end of day, together with the observed incidence and 95% confidence interval of CIs and CIEs in each material:

<table>
<thead>
<tr>
<th>Material</th>
<th>Total No. Doses</th>
<th>Contact on Insertion</th>
<th>Contact Post-Dose</th>
<th>Change at End of Day</th>
<th>Observed Incidence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>102</td>
<td>51.0 (40.4-61.6)</td>
<td>5.3 (4.3-6.3)</td>
<td>4.8 (3.8-5.8)</td>
<td>0.00 (0.0-0.0)</td>
<td>0.00</td>
</tr>
<tr>
<td>DD+PS</td>
<td>116</td>
<td>51.0 (40.4-61.6)</td>
<td>5.3 (4.3-6.3)</td>
<td>4.8 (3.8-5.8)</td>
<td>0.00 (0.0-0.0)</td>
<td>0.00</td>
</tr>
<tr>
<td>DD+H/A</td>
<td>83</td>
<td>51.0 (40.4-61.6)</td>
<td>5.3 (4.3-6.3)</td>
<td>4.8 (3.8-5.8)</td>
<td>0.00 (0.0-0.0)</td>
<td>0.00</td>
</tr>
<tr>
<td>DD+Verde</td>
<td>80</td>
<td>51.0 (40.4-61.6)</td>
<td>5.3 (4.3-6.3)</td>
<td>4.8 (3.8-5.8)</td>
<td>0.00 (0.0-0.0)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Higher scores indicate less comfort or more symptoms.
CLAY Study Results
n= 3,549 with 187 Infiltrative Events

- Increased risk of Infiltrates for:
  - Young age approx 2X (but not children)
  - Non-linear, peak @ 15-25 yrs
  - Extended wear (2.4X, 1.7, 3.3)
  - SiHys (1.8X, 1.3, 2.7)
  - Multi-Purpose LCPs (2.9X, 1.3, 6.2)
  - H2O2 referent
  - No MPS brands named
  - < 1 Yr SCL wear is protective

Szczotka-Flynn et al Optom & Vis Sci 2014

Corneal Inflammatory Events with Daily Silicone Hydrogel Lens Wear

Szczotka-Flynn et al Optom & Vis Sci 2014
How often are lenses, storage cases, and lids contaminated during Silicone Hydrogel Daily Wear?

<table>
<thead>
<tr>
<th></th>
<th>Number of Subjects with at least one culture positive result (%)</th>
<th>Number of Subjects with at least two culture positive results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lens (n = 196)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant bioburden</td>
<td>142 (72.8%)</td>
<td>23 (11.8%)</td>
</tr>
<tr>
<td>Lid (n = 217)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant bioburden</td>
<td>53 (24.4%)</td>
<td>86 (39.6%)</td>
</tr>
<tr>
<td>Conjunctive (n = 217)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant bioburden</td>
<td>147 (67.7%)</td>
<td>30 (13.8%)</td>
</tr>
<tr>
<td>Case (n = 174)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant bioburden</td>
<td>53 (30.5%)</td>
<td>90 (50.2%)</td>
</tr>
<tr>
<td>Transport saline (n = 195)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant bioburden</td>
<td>118 (60.5%)</td>
<td>43 (22.1%)</td>
</tr>
<tr>
<td>Lens or Transport saline (n = 180)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant bioburden</td>
<td>105 (53.8%)</td>
<td>61 (31.3%)</td>
</tr>
</tbody>
</table>

Contact Lens Case Contamination during Daily Wear of Silicone Hydrogels
Bacterial diversity in Storage Cases of CIE patients

- One MPDS represented
- Role of Gram Negatives, endotoxin
  - Achromobacter spp. ~60%
  - Stenotrophomonas maltophilia 22%
  - Serratia marcescens and Delftia spp 17%
  - Elizabethkingia spp. and Chryseobacterium indologenes 11%

Kilvington et al CLAE 2013

Microbiome Abundance on Worn Contact Lenses

Loretta Szczotka-Flynn OD, PhD
Mauricio Retuerto MS
Pranab Mukherjee PhD
Jyotana Chandra PhD
Ying Jiang MS
Sara Debanne PhD
Mahmoud Ghannoum PhD
Sponsor: Alcon Research Ltd

Background

- Corneal infiltrative events (CIEs) are associated with bacterial bioburden of the eye and lens surfaces.
- Microbes can reside on lenses and within lens cases, often in association with multi-species biofilms that serve as reservoirs for the establishment of corneal infection and inflammation.
- More than 50% of worn contact lenses and between 24–81% of contact lens storage cases are contaminated with microbes. (Szczotka-Flynn, Ghannoum, Pearlman 2010)
- Next-generation sequencing provides the ability to identify microbes adhering to contact lenses.
How We Study the Human Microbiome

Assessing the Human Microbiome

• The Human Microbiome Project established a comprehensive catalog of microbial organisms living in or on the human body.
• Modern high speed nucleic acid sequencing can fully characterize all commensal and pathogenic live or dead species that inhabit the human body including the ocular surface - or contact lenses sitting on the ocular surface.
  – One common technique is to sequence a marker: a short, unique DNA sequence that can be used to identify the genome that contains it
• One common DNA marker is the gene that codes for the 16S subunit of ribosomal RNA, an important part of the cell's protein-building machinery.

Other studies on the ocular microbiome

IOVS 2011

211 species level phylotypes per subject
31% belonged to unclassified, novel bacteria
12 genera ubiquitous including Pseudomonas, Propionibacterium, Corynebacterium, Pseudomonas, Staphylococci, Streptococci, and others
Other studies on the ocular microbiome:

**Bacterial diversity in Storage Cases of CIE patients**

- Culture independent method
  - 16S ribosomal RNA (rRNA) gene analysis
- Increase in PCR amplifiable products from storage cases of patients with keratitis (vs controls)
  - Consistent with theory that bacterial contamination of lens cases is associated with contact lens-related disease.
- Bacterial diversity is associated with severity of contact lens-related disease

*Wiley IOVS, 2012*

---

**Purpose:**

- To assess the microbiome adherent to lotrafilcon A contact lenses worn during asymptomatic daily wear
- To define and compare bacterial communities on lenses from patients associated with successful wear and eventual corneal inflammatory events (CIEs)

---

**METHODS**

- All subjects participated in the Daily Wear Corneal Infiltrative Event (DWCIE) Study:
  - Lotrafilcon A lenses worn daily wear with monthly disposal
  - Randomized to peroxide (Clear Care) vs. a PHMB-preserved MPS (Renu Fresh)
  - Followed for 1 year
  - Lenses collected at the 2 month visit for microbiome analysis
  - Lens from one eye of each patient used
  - Lenses collected aseptically during period of successful wear, prior to any CIE event, stored frozen in 1 ml non-preserved saline
- 21 worn CLs from asymptomatic subjects were screened for bacterial presence using 16s rRNA molecular amplification
  - 14 CLs from CIE-free subjects
  - 7 CLs from subjects that developed a CIE over 1 year of follow-up
  - Controls 2:1 matched on age (±5 years), solution, sex, and ethnicity to the cases and randomly chosen from those that successfully completed the study without a CIE.
• Cohort Characteristics
  • Age range 21-41
  • CIE Cases:
    – 5 females, 2 males
    – 5 Clear Care Users, 2 Renu Multiplus Users
    – 3 Asian, 4 Caucasian
  • Controls:
    – 10 females, 4 males
    – 10 Clear Care Users, 4 Renu Multiplus Users
    – 3 Asian, 11 Caucasian

METHODS
• 16S rRNA amplicon was identified in 20/21 (95%) of contact lenses; the
  amplification product was subjected to next generation sequencing via
  the ION torrent PGM workflow.
  – Lens assessed from CIE subjects was NOT the lens associated with
    the event
  – The lens assessed was worn during a period of successful wear prior
    to the event
• Bioinformatics was performed using Qlime Platform (ver. 1.8) and the
  Greengene (ver. 13.8) reference database.
• Outcomes within the covariates were analysed using Cluster Analysis
  (Principle Component Analysis) and Kruskal-Wallis tests.
128 different genera defined the lens biome with relative abundance >0.01%

- Lens biome was defined by 252,539 sequences, with an average of 20,044 sequences per sample and an average read length 230 bp.
- Bacterial diversity on CLs of CIE-free subjects was greater than CIE-subjects

CIE (n=7) 139 Genera
Control (n=14) 154 Genera
49 Genera unique to control lens
34 Genera unique to CIE prone lens

Bacteria defined by >1% relative abundance were common to both groups and included Ralstonia, Streptococcus, Corynebacterium, Stenotrophomonas, Delftia, Enterococcus, Staphylococcus, Acinetobacter, Propionibacterium, Halomonas, Pseudomonas, and Actinomyces.

Only Acinetobacter showed a difference in abundance between the 2 groups (greater abundance in CIE-free subjects, P=0.007).
Conclusion

- Worn CLs harbour an unexpectedly diverse microbial community
  - Commensal, environmental, and pathogenic bacteria found on lenses during successful wear include:
    - Gram Positives:
      - Streptococcus, Corynebacterium, Enterococcus, Staphylococcus, Propionibacterium, Actinomyces
    - Gram Negatives:
      - Ralstonia, Stenotrophomonas, Delftia, Acinetobacter, Halomonas, Pseudomonas
  - When differences were found in the lens biome, CLs from CIE subjects (during periods of successful wear) almost always harboured greater relative bacterial abundance.

Questions, Future Directions

- Does the increased abundance on lenses of CIE subjects suggest a carrier effect?
  - Opposite to traditional thought that CIE related organisms were transient and sporadic (Keay 2001, Szczotka-Flynn 2009, Gopinathan 1997)

- Does the decreased diversity and increased abundance in CIE subjects suggest a disruption of the symbiotic relationships of the host biome?
  - Could this altered biome predispose patients to intermittent, substantial bacterial contamination contributing to CIEs?

- Future prospective studies should use culture-free, modern high speed DNA sequencing methods when assessing lens related bioburden in association with CIEs
  - Repeated assessment prior to and at the time of CIE vs controls

THANK YOU
**Central corneal stroma 24h after topical exposure to LPS**

**Normal C57Bl/6**

**LPS treated C57Bl/6**

Infiltrates evident, highly refractile cells

---

**Bacterial diversity on contact lenses of patients wearing daily wear SH lenses**

75% (64/84) of contact lenses bore 16s rRNA and the amplification product underwent next generation sequencing via the ION torrent PGM workflow

Len Microbiome (n=64) Dominant Genera

---

Source: RETUERTO, SZCZOYKA-FLYNN ET AL. ARVO 2014

---
CLs stored in peroxide was lower than MPS (94 vs. 153 genera) with 12 bacteria unique to peroxide vs. 71 unique to MPS.

**Lens Microbiome Diversity based on Care Solution Used**

![Diagram showing genera in storage solutions for peroxide and MPS]

*Cases stored in peroxide was lower than MPS (94 vs. 153 genera) with 12 bacteria unique to peroxide vs. 71 unique to MPS*

Source: RETUERTO, SZCZOTKA-FLYNN ET AL, ARVO 2014

---

Relative abundance comparisons indicated *Corynebacterium*, *Streptococcus*, *Aggregatibacter*, *Peptoniphilus* and *Haemophilus* had higher abundance in MPS users (all p <0.05)

**Lens Microbiome Abundance**

![Diagram showing abundance in storage solutions for Gram (+) and (-)]

Source: RETUERTO, SZCZOTKA-FLYNN ET AL, ARVO 2014

---

**Summary**

- Incidence of contact lens related infiltrates during daily wear (i.e. in association with solution use) ~3-7%
  - Regional differences may exist
- Bacteria likely causative for CIE in DW as is it well known in EW
  - Storage case contamination widespread
- Culture independent methods to identify bacterial abundance and diversity may explain impact of care solutions on CIEs
  - Increased bacterial diversity and abundance on MPS stored lenses vs. peroxide may explain increases in CIE in MPS users
A Prospective, Randomized, Open Label Clinical Trial on the Association Between Mucin Balls and the Development of Corneal Infiltrative Events during Extended Wear With Silicone Hydrogel Contact Lenses

SPONSOR: VISTAKON, JOHNSON & JOHNSON VISION CARE, INC.

Study Design and Results

Co-investigators: Sara Debanne PhD, Ying Yiang, MS, Mary Jo Steigeimeir OD, Jeff Walline OD, PhD, Don Mutti OD, PhD, Tawnya Wilson OD

STUDY BACKGROUND, OVERVIEW & DESIGN

Loretta Szczotka-Flynn OD, PhD
Professor; Departments of Ophthalmology & Visual Sciences and Epidemiology & Biostatistics; Case Western Reserve University
Director Contact Lens Service; University Hospitals of Cleveland
BACKGROUND

THE LONGITUDINAL ANALYSIS OF SILICONE HYDROGEL (LASH) CONTACT LENS STUDY

- **Microbial contamination**
- Presence of ocular surface disruption (staining or disrupted mucins)

**CIE Conceptual Model:**
The development of a CIE is likely related to a sequence of events including increased bacterial loads on lens surfaces with a breakdown of ocular defense barriers.

---

**THE LASH STUDY**

**Number (percentage) of subjects with culture positive lenses stratified by visit and presence of infiltrate**

<table>
<thead>
<tr>
<th>No Infiltrative Event</th>
<th>During Infiltrative Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk post IV visit</td>
<td>4 month post IV visit</td>
</tr>
<tr>
<td>10 (12.1%)</td>
<td>15 (12.0%)</td>
</tr>
<tr>
<td>20 (44.4%)</td>
<td>8 (52.6%)</td>
</tr>
<tr>
<td>Any event</td>
<td>Asymptomatic Events</td>
</tr>
<tr>
<td>27 (10.5%)</td>
<td>14 (12.5%)</td>
</tr>
<tr>
<td>Symptomatic Events</td>
<td></td>
</tr>
<tr>
<td>10 (5.8%)</td>
<td>8 (4.2%)</td>
</tr>
<tr>
<td><em>p-value compared to asymptomatic events</em></td>
<td></td>
</tr>
</tbody>
</table>

---

**THE LASH STUDY**

Unadjusted cumulative probability of remaining CIE free stratified by presence or absence of at least one episode of moderate corneal staining or greater

Log Rank Test: $p = 0.9348$
THE LASH STUDY

Unadjusted cumulative probability of remaining CIE free stratified by presence or absence of substantial bioburden on study lenses

Hazard Ratio
8.66 (p<0.0001)

Log Rank Test:
p=0.0254
HR = 4.13

THE LASH STUDY

Unadjusted cumulative probability of remaining CIE free stratified by smoking status over 1 year of follow-up

Univariate Hazard Ratio
0.17 (95% CI 0.06-0.43)
Risk Factor Analysis for CIE

- **Corneal Staining**: Not associated with bacterial contamination
- **Smoking**: 400% increased risk
- **Mucin Balls**: 84% decreased risk

**THE LASH STUDY**

Grading of Mucin Ball Presence by Fluorescein Evaluation and Frequency of Occurrence Across 1047 subject-visits in LASH cohort

<table>
<thead>
<tr>
<th>Any reportable presence</th>
<th>Substantial Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zones</td>
<td>Surface Area</td>
</tr>
<tr>
<td>n=11</td>
<td>n=2</td>
</tr>
</tbody>
</table>

- Frequency of subject visits
  - Any reportable presence: 54.8%
  - Substantial presence: 10.0%

1. Masked reader data
2. Investigator data

**THE LASH STUDY**

Frequency of Mucin Ball Presence by Visit

<table>
<thead>
<tr>
<th>1 week post</th>
<th>1 month post</th>
<th>4 month post</th>
<th>8 month post</th>
<th>12 month post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visits</td>
<td>Visits</td>
<td>Visits</td>
<td>Visits</td>
<td>Visits</td>
</tr>
<tr>
<td>n=150</td>
<td>n=150</td>
<td>n=127</td>
<td>n=84</td>
<td>n=85</td>
</tr>
<tr>
<td>Any mucin ball presence</td>
<td>26 (34%)</td>
<td>30 (32%)</td>
<td>31 (28%)</td>
<td>32 (34%)</td>
</tr>
<tr>
<td>Substantial mucin ball presence</td>
<td>16 (20%)</td>
<td>24 (25%)</td>
<td>31 (27%)</td>
<td>58 (69%)</td>
</tr>
</tbody>
</table>

*masked reader data
Multivariate Analysis for Development of CIE

Model 1

<table>
<thead>
<tr>
<th>Model</th>
<th>Factor</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Mucin Ball Presence</td>
<td>1.10</td>
<td>0.90-1.35</td>
</tr>
<tr>
<td>Model 2</td>
<td>Mucin Ball Presence</td>
<td>1.10</td>
<td>0.90-1.35</td>
</tr>
</tbody>
</table>

In the LASH Study, repeated presence of mucin balls was associated with an 82-84% decreased hazard of a CIE.

THEORY:

Mucins in healthy tears

- At least 4 of 19 mucin genes found on the ocular surface
  - Soluble MUC5AC secreted by goblet cells for viscosity
  - Membrane spanning mucins
    - MUC1 and MUC16 secreted by corneal and conjunctival epithelium
    - MUC4 secreted by conjunctival epithelium
- May represent a different mucin profile which renders a subject less likely to upregulate the immune response against bacterial ligands
- OR
- Mucin Balls themselves are a physical barrier between the eye and contact lenses preventing upregulation of the immune system
Mucin Ball Study

This study was designed to determine the influence of early MB formation via a provocative run-in phase vs. longer term physical presence of MBs on the rate of CIEs while controlling for lens type and bacterial bioburden during extended wear.

Hypotheses:
- 1. The physical presence of mucin balls during extended wear with silicone hydrogel lenses offers protection against CIEs.
- 2. The ability to form mucin balls offers protection against CIEs during extended wear with silicone hydrogel lenses.

Mucin Ball Study Design

A randomized clinical trial (RCT) was nested within a cohort study.

RCT answers Hypothesis 1
- Two lenses of different modulus used to determine if the physical presence of mucin ball formation is associated with a CIE
  - comfilcon A (Biofinity)
  - balafilcon A (PureVision)
- Blocked randomization by site and the ability to form mucin balls
- Subjects followed over 12 months of extended wear for the development of a CIE
- The repeated presence of mucin balls in the RCT is the main covariate of interest

Cohort study answers Hypothesis 2
- Subjects are self-selected as mucin ball formers, or not, after approximately a 1 month run-in phase comprised of up to 30 days of continuous wear with the lotrafilcon A lens (Air Optix Night & Day Aqua)
- Ability to repeatedly form mucin balls is main covariate of interest

Study Sites

3 sites participated in the USA
- University Hospitals Case Medical Center
  - PI Loretta Szczotka-Flynn OD, PhD
  - Cleveland, Ohio
- The Ohio State University College of Optometry
  - PI Don Mutti OD, PhD and Jeff Walline OD, PhD
  - Columbus, Ohio
- Western Reserve Vision Care
  - PI Mary Jo Begenstein OD
  - Beachwood, Ohio
Mucin Ball Definitions

- MB formation in each phase was defined as repeated presence of any MBs, on a person-level.
  - MB formation was assessed from white light slit lamp examination with lenses worn, and by MB induced impressions detected by fluorescein administration after lens removal.
Grading Mucin Ball Depressions

Depressions from mucin balls: pool fluorescein post lens removal

Comparison of corneal staining and mucin ball depressions
Statistical Analysis

- Multivariable Cox proportional hazards regression was used to model the probability of a CIE as a function of MB formation and other key covariates.

RESULTS

- 282 subjects entered into Phase 1
  - mean age 34 years
  - 65% female
  - 7.8% neophytes
  - 72% Caucasian, 8.5% Asian eye
  - 63 subjects were lost to follow up, discontinued or had a CIE during Phase 1

- 219 subjects entered into Phase 2
  - mean age 34 years
  - 64% female
  - 6% neophytes
  - 70% Caucasian, 8% Asian eye
  - Among them 35 CIEs occurred
Hypothesis 1

- The physical presence of mucin balls during extended wear with silicone hydrogel lenses (Phase 2) offers protection against CIEs
  - 10.5% of MB formers experienced a CIE
  - 14% of non-MB formers experienced a CIE

### CIE Rate by MB presence in Phase 2

- **Phase 2 repeat MB former**
  - N=153 (70%)
  - CIE rate= 10.5% (16/153)

- **Phase 2 NON-repeat MB former**
  - N=43 (20%)
  - CIE rate= 14.0% (6/43)

### PureVision

- N=66 (43%)
  - CIE rate= 4.5% (3/66)

### Biofinity

- N=87 (57%)
  - CIE rate= 14.9% (13/87)

- **Phase 2 non-MB former**
  - N=43 (20%)
  - CIE rate= 14.0% (6/43)

- **MB former**
  - N=153 (70%)
  - CIE rate= 10.5% (16/153)

### Univariate Kaplan-Meier curve for CIEs by repeated MB presence in Phase 2
Hypothesis 1

Repeated, long term MB presence during wear of balaficon A or comficon A lenses over 1 year of follow-up significantly decreased the hazard for developing CIEs by 62%.

Hazard Ratio (HR) 0.380, p=0.0494

Hypothesis 2

• The ability to form mucin balls (in Phase 1) offers protection against CIEs during extended wear with silicone hydrogel lenses.
  - 19% of MB formers in the run-in phase experienced a CIE in the 12 month follow-up phase
  - 6% of non-MB formers in the run-in phase experienced a CIE in the 12 month follow-up phase
CIE Rate in Phase 2 by MB Formation in Phase 1

Univariate Kaplan-Meier curve for CIEs by repeated MB presence in Phase 1

Multivariate Survival Analysis Model for CIE in Phase 2

covariate of interest is MB presence in Phase 1

Conclusions

• The overarching hypothesis that MB formation is protective against CIEs throughout EW is not supported.
  – Hypothesis 1
    • A protective effect of longer term MB presence on rate of CIEs was detected
    • Statistically significant decreased hazard (by 62%) for developing CIEs over 1 year
    • Same finding as LASH Study
  – Hypothesis 2 was not supported.
    • The ability to form MBs in the provocative 1 month run-in (Phase 1) did not offer protection against CIEs (Phase 2) and resulted in the opposite effect.
    • Statistically significantly increased hazard of CIEs (by 470%) for MB formers compared to the non-MB formers determined in Phase 1
  – Early onset MB formation substantially increases the hazard for CIE in subsequent wear with different lens types.

Thoughts

• Early MBs may signify a change to the mucin layer
  – Precipitate CIE through the disruption of an important barrier defense against bacterial antigens.
  – Shed mucins can regenerate, thus not necessarily an immediate CIE response
• Grupcheva et al: dendritic cells accumulate in the anterior corneal stroma beneath MBs on confocal microscopy
  – Dendritic cells prime the immune response for recurrent clinical inflammatory events under the right conditions
• Ladage et al: MBs activate stromal keratocytes immediately beneath the MB embedded in the epithelial surface
  – Heightened immune response primes the immune system for later activation when challenged

Thoughts

• Low grade - chronic presence - of MBs can also serve a protective role
  – physical barrier between the lens and ocular surface
  – not be enough to disrupt the mucous layer
  – instead acts as a ball bearing effect on the ocular surface
CORNEAL TRANSPLANTATION

Types of Corneal Transplants
- Penetrating Keratoplasty (PKP or PK)
- Deep Anterior Lamellar Keratoplasty (DALK)
- Endothelial Keratoplasty (EK)
  - DSEK
  - DSAEK
  - DMEK

TYPES OF TRANSPLANTS

Full thickness PK  DALK  DSAEK
Deep Anterior Lamellar Keratoplasty (DALK) is the selective manual replacement of 99% thickness of the anterior corneal tissue, leaving only recipient Descemet's membrane as the recipient bed.

Main indications for DALK (any corneal disease with healthy endothelium):
- Corneal scars with healthy endothelium
- Herpes Simplex scarring
- Herpes Zoster scarring
- Ectasia following Lasik surgery
- Ectasia following RK surgery
- Atopic Disease
- Down's Syndrome patients
- Deeply vascularized corneas

Dystrophy Eyes that benefit from DALK:
- Keratoconus without Hydrops
- Central Crystalline Dystrophy
- Lattice Stromal Dystrophy
- Granular Stromal Dystrophy
Two Basic Approaches to DALK

- Limbal pocket dissection to near Descemet's membrane (Melles)
- Total dissection with baring of Descemet’s membrane (Anwar, Shimmura, Sugita)

Advantages of DALK over PK

- Safer procedure: no open sky
- Retained Recipient Endothelium: no late endothelial cell loss
- No significant rejection issues
- Patient off steroids in matter of weeks, not years
- Sutures can be removed in only weeks after surgery
- Less risk of rupture in future
- Especially great procedure for Atopic pts, Downs syndrome pts, vascularized recipient rim eyes

Disadvantages of DALK to PK

- Less percentage of eyes attain BCVA of 20/20 with DALK than PK eyes, but an equal percentage of 20/25
- DALK is much more technically demanding than PK and takes more O.R. time
Endothelial Keratoplasty: Multiple names and acronyms

- PLK: Posterior Lamellar Keratoplasty (Melles)
- DLEK: Deep Lamellar Endothelial Keratoplasty (Terry)
- DSEK: Descemets Stripping Endothelial Keratoplasty (Price)
- DSAEK: Descemets Stripping with Automated Endothelial Keratoplasty (Gorovoy)
- DMEK: Descemets Membrane Endothelial Keratoplasty (Melles)
- DMAEK: Descemets Membrane Automated Endothelial Keratoplasty (Price)

Interface fluid resolves without intervention
1 day post-DSAEK
UCVA=20/200

1 week post-DSAEK
UCVA=20/200

3 weeks post-DSAEK
UCVA=20/100

2 months post-DSAEK
+1.25 + 1.00 x 25 = 20/40
UCVA=20/60

1 Wk PO, Superior wedge cleft
Small clefts

Re-bubble at one week  
1 month post-op

Graft Rejection Assessment

Types of Clinical Rejection Episodes
- Epithelial rejection
- Subepithelial Infiltrates
- Stromal
- Endothelial
Endothelial Rejection

- Most severe
- Signs
  - Khodadoust Line
  - Diffuse
    - KPs on donor endothelium

Probable corneal rejection episode

- Hazy graft that was previously clear
- Corneal edema
- Inflammation
  - Stromal infiltrates
  - KPs
  - AC cells
  - Ciliary injection

CPTS Graft Rejection Classification

Possible/Probable

- Possible presence of a new keratic precipitate(s) with difficulty distinguishing between keratic precipitate vs. pigment

Courtesy of A. Aldave
CPTS Graft Rejection Classification

Definite: Mild

- Presence of one or more of the following signs:
  - one to five keratic precipitates,
  - increase in aqueous cells from previous visit without clinically apparent change in stromal thickness from previous visit or clinically evident stromal and/or epithelial edema.

The management of suspected graft rejection episodes will be according to the investigator prerogative, but documented in the medication history.

Definite: Severe

- Presence of one or more of the following signs:
  - more than five keratic precipitates,
  - cells in the stroma,
  - endothelial rejection line,
  - moderate to severe increase in aqueous cells from previous visit; or
  - any of the above with or without clinically apparent change in recipient stromal thickness associated with stromal and/or epithelial edema from previous visit and change in stromal clarity.

The management of suspected graft rejection episodes will be according to the investigator prerogative, but documented in the medication history.
CPTS Graft Rejection Classification

Definite: Severe

- Presence of one or more of the following signs:
  - more than five keratic precipitates,
  - cells in the stroma,
  - endothelial rejection line,
  - moderate to severe increase in aqueous cells from previous visit; or
  - any of the above with or without clinically apparent change in recipient stromal thickness associated with stromal edema.

Definite Severe Graft rejection post EK

Principal Causes of Graft Failure

- Early failure (cloudy cornea on the first postoperative day which does not clear or requires a regraft within 8 weeks), associated with surgical complications.
- Primary donor failure (cloudy cornea on the first postoperative day which does not clear or requires a regraft within 8 weeks), in the absence of surgical complications.
- Graft rejection (defined as a clouded recipient central stroma following an allograft reaction).
- Non-rejection graft failure (defined as a graft that initially had a clear central recipient stroma and becomes cloudy due to causes other than an immune event. These include: surface failure, infection, glaucoma/hypotony, endothelial decompensation, interface irregularity or opacity, stromal scar, blunt or penetrating trauma, and other causes).
- Refractive/visual graft failure (defined as a graft that requires regrafting due to inadequate vision while the recipient central stroma remains clear).
**Principal Causes of Graft Failure**

**Early Failure**

- Early failure (cloudy cornea on the first postoperative day which does not clear or requires a regraft within 8 weeks), associated with surgical complications.

**Primary Donor Failure**

- Primary donor failure (cloudy cornea on the first postoperative day which does not clear or requires a regraft within 8 weeks), in the absence of surgical complications.

**Graft Rejection**

- Graft rejection (defined as a clouded recipient central stroma following an allograft reaction);
Principal Causes of Graft Failure

Non-rejection Graft Failure

- Non-rejection graft failure (defined as a graft that initially had a clear central recipient stroma and becomes cloudy due to causes other than an immune event. These include: surface failure, infection, glaucoma/hypotony, endothelial decompensation, interface irregularity or opacity, stromal scar, blunt or penetrating trauma, and other causes).

Principal Causes of Graft Failure

Refractive/Visual Graft Failure

- Refractive/visual graft failure (defined as a graft that requires regrafting due to inadequate vision while the recipient central stroma remains clear).

CPTS Recipient Corneal Stroma Clarity

Grading Scale for Endothelial Keratoplasty
NIH Funded Large Scale Studies on Corneal Transplantation

- Collaborative Corneal Transplant Studies (CCTS)
- Cornea Donor Study (CDS)
- Corneal Preservation Time Study (CPTS)
  - Collectively explored in association with graft success:
    - Tissue/histocompatibility matching (CCTS)
    - ABO blood type matching (CDS)
    - Donor age (CDS)
    - Donor Preservation Time (CPTS)

**Collaborative Corneal Transplantation Studies**

- Between 1986 and 1989, CCTS Group conducted two controlled, double-masked studies addressing donor-recipient histocompatibility matching.
- After 3 years of patient follow-up, participants that received corneal transplants with well-matched antigens did not fare significantly better than those with a poor match.
  - Each patient group had similar rates of initial immune reactions, graft rejection, and graft failure due to rejection or other causes.
  - However, the researchers did note that CCTS patients who were compatible with the donor's blood type had a better outcome than unmatched patients.

- In short, data from the CCTS indicated that matching patient and donor blood types (combined with treating patients with high-dose topical steroids after surgery) may be potentially effective in improving high-risk corneal transplantation.
Purpose of the Cornea Donor Study

Objective: to determine whether donor age is associated with corneal transplant success

Rationale:
- potential for the donor pool to decrease in the future
- limited, inconclusive scientific literature on the suitability of older donor tissue
- international demand for donor tissue
- limited longitudinal data on graft survival rates

Study Design

Cornea Assignment:
- Corneas assigned from donor ≥66 and from donor <66 using a random approach without respect to recipient factors

Masking:
- Investigator and patient masked to age of donor tissue

Treatment:
- Surgery and postoperative care by surgeons’ usual routine

Study Outcome:
- Graft failure based on clinical exam during 5 year follow up
Key Recipient Eligibility Criteria

- Age 40 to 80 years
- Corneal disease associated with endothelial dysfunction (moderate risk for failure)

Key Donor Eligibility Criteria

- Age 10 to 75 years
- Endothelial cell density 2300-3300
- Eye Bank Association of America criteria met

Enrollment and Participation

- 1,101 subjects enrolled January, 2000 to August, 2002
  - 11 subjects with ineligible diagnoses
  - 1,090 eligible subjects
- 43 eye banks provided corneas to CDS subjects
- 105 surgeons at 80 sites enrolled subjects
Subject Demographics (N=1090)

- Mean Age (range) 70 (41 - 86) years
- Female 64%
- Caucasian 93%

Donor Age

- Mean = 58 years
- Range 12 to 75 years

- Cornea from donor ≥ 66 yrs (35%) 383
- Cornea from donor < 66 yrs (65%) 707

5-Year Graft Success Rates

Similar graft success rates
- Donor Age ≥ 66 years 86%
- Donor Age < 66 years 86%

Difference = 0%
  - Limit of one-sided 95% CI = 4%
  - Less than pre-specified non-inferiority limit of 8%
Graft Success by Donor Age Group

5-Year Graft Success by Donor Age

Donor Factors Associated with Graft Failure

- Graft failure rates were not significantly impacted by:
  - any donor characteristics
  - any factors related to the type of tissue retrieval, processing, timing of use of the cornea
  - any characteristics of the donor cornea
- Adjusting for donor age did not affect the results.
Has the CDS had an impact on the acceptance of corneas from older donors?

Donors Used for Transplant in the US
Complete Data for 7 Eye Banks

<table>
<thead>
<tr>
<th></th>
<th>≥ 66 years</th>
<th>Median Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before CDS (1998-1999)</td>
<td>19%</td>
<td>53 (39-63)</td>
</tr>
<tr>
<td>During CDS (2000-2007)</td>
<td>21%</td>
<td>54 (41-64)</td>
</tr>
</tbody>
</table>

all p < 0.001

Endothelial Cell Density Determination

- Variable Frame Analysis
- Independent ECD determination by 2 certified readers
- Adjudication if ECD determined by 2 readers differed by ≥ 5%
Endothelial Cell Density over 5 Years by Donor Age Group
(only includes subjects with graft success through 5 years)

Percent Endothelial Cell Loss from Baseline to 5 Years
Spearman Correlation Coefficient (95% CI) = −0.20 (−0.30, −0.09)

Conclusions
- 5-yr graft success rate similar with corneas from donors ≥66 yrs and <66 yrs old
- Suggestion of a slightly higher success rate with very young donors
- Endothelial cell loss is substantial over 5 yrs even with successful transplant
- Slightly greater cell loss in corneas from donors ≥66 yrs than <66 yrs old
Cornea Preservation Time
Advantages of extending beyond 7-8 days

- Improve efficiency of tissue distribution and reduce need for export
- Respond to future threats to the donor pool related to increase domestic demand and tissue spoilage from EK donor preparation
- Provide more time for the eye banks to conduct tissue evaluation with anticipated more extensive testing for emerging infections and increased regulations occurring over next 10 years with an increasingly impaired donor pool
- Evidence of a lower graft rejection rate with longer preservation time

Cornea Preservation Time Study (CPTS) Objectives

- To determine if the 3-year graft failure rate following EK performed with donor corneas with a preservation time of 8 to 14 days is non-inferior to the failure rate when donor corneas with a preservation time of 7 or fewer days were used.
- To determine if the central corneal endothelial cell density 3 years after EK is related to preservation time.
- To evaluate donor, operative and postoperative factors on graft failure and endothelial cell density three years following EK.

Why Cornea Preservation Time and a multicenter prospective randomized clinical trial opportunity

- US surgeons reluctant to use donor tissue beyond 7-8 days from death to surgery
- Limited clinical studies in the United States using donor corneas for PK or EK with extended time out to FDA-approved time in storage of 14 days for Optisol GS at 4°C and none for recently released Life 4°C
- Excellent, but uncontrolled, experience with exported tissue internationally with use of tissue beyond 7 days
Methods

- CPTS and non-CPTS eye banks were queried for data on each cornea for DSEK in 2010-2011, prior to initiation of CPTS enrollment in April 2012.
- Data collected for each case:
  - USA or international placement
  - Whether precut by eye bank
  - Donor age
  - Cause of death
  - Dates of death, preservation, and surgery
- Data collection will be repeated at the end of recruitment (2014) and after study results are published (2017).

Preservation Time
Domestic vs International

<table>
<thead>
<tr>
<th>Days from Preservation to Surgery</th>
<th>Domestic (N=6632)</th>
<th>International (N=9231)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 days</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>5-7 days</td>
<td>46%</td>
<td></td>
</tr>
<tr>
<td>8-11 days</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>12-14 days</td>
<td>&lt;1%</td>
<td></td>
</tr>
</tbody>
</table>

Median (IQR) Preservation Time:
- Domestic: 4 (3, 6)

Preservation Time
Domestic

<table>
<thead>
<tr>
<th>Days from Preservation to Surgery</th>
<th>Preservation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 days</td>
<td>92%</td>
</tr>
<tr>
<td>5-7 days</td>
<td>46%</td>
</tr>
<tr>
<td>8-11 days</td>
<td>2%</td>
</tr>
<tr>
<td>12-14 days</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

Median (IQR) Preservation Time: 4 (3, 6)
Preservation Time (Domestic)
2010 vs 2011

Conclusions
CPTS Eye Bank Impact Study
2010-2011 Domestic Data

- 98% of donor corneas placed for DSEK were preserved 7 days or less
- Preservation time was associated with cutting source, year, eye bank.
  - Although differences were statistically significant, they may not be meaningful in a practical sense
- Great opportunity to change practice patterns regarding preservation time in the United States if show no difference in graft success and cell density at 3 years with the donors over 7 days

Preliminary AR Rates Comparison

<table>
<thead>
<tr>
<th>Eye Bank</th>
<th>D-5 Over Seven Days</th>
<th>All Cornea Grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grafts</td>
<td>AR</td>
</tr>
<tr>
<td>SightLife</td>
<td>1,212</td>
<td>1</td>
</tr>
<tr>
<td>Heartland Lions Eye Banks</td>
<td>995</td>
<td>-</td>
</tr>
<tr>
<td>Midwest Eye Banks</td>
<td>1,032</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>3,239</td>
<td>3</td>
</tr>
</tbody>
</table>
Treatment groups, sample size, recruitment period and follow up

- Study eyes will be randomly assigned to receive either a donor cornea
  - With a preservation time of 8 to 14 days or
  - With a preservation time of 7 or fewer days.
- All available donors will be listed on the study website in the appropriate preservation time group and computer algorithm will select donor assignment
- Study staff and participant will be masked as to all donor parameters except the preservation medium
- Sample size: 1330 eyes
- Recruitment period: 16 months starting March 1, 2012
- Follow up: Three years

Cornea Preservation Time Study (CPTS)

Donor criteria

- Meets current EBAA standards
- Corneas suitable for EK (eye bank prepared according to normal procedure for the surgeon or surgeon-prepared)
- Donor age at death 10 to 75 years
- Death to preservation time: ≤20 hrs if body refrigerated or eyes on ice within 10 hours of death and ≤10 hrs if not refrigerated for both groups

CPTS Clinical Sites

[Map of CPTS Clinical Sites]
Thank You