Preservation of Corneal Grafts
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Disclosure Statement:
• Nothing to disclose

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Background
• Corneal grafts are the first successful solid tissue transplants in medicine
• They are the most common form of tissue transplantation in medicine
• They are the most successful tissue transplant techniques in medicine
• Doesn’t necessarily mean their management is straightforward

Optometric Education on Keratoplasty....
Corneal Transplantation and Optometry

Confusion

Outline

• Today’s goals:
  • Reduce the confusion
    – Facilitate understanding of the physiologic/immunologic implications of keratoplasty
    – Facilitate understanding of the language of keratoplasty
    – Increase awareness of newer surgical techniques and approaches to graft preservation
    – Increase comfort with working with grafts clinically
Keratoplasty Mental Exercise

• What is meant by immune privileged?
• What is meant by graft rejection?
• What is the appropriate treatment of rejection?
• If endothelium is healthy but a patient needs a transplant, what are the benefits of maintaining host endothelium?
• What are the benefits to a sutureless transplant?

Cornea: A review

• Function: Optical and structural
• Five (or is it six...) layers thick:
  – Epithelium, Bowman’s Membrane, Stroma, (Oua’s Layer), Descemet’s Membrane and endothelium
  – Cellular reservoirs are the epithelium, stroma (keratocytes) and endothelium
  – Corneal substance is primarily acellular connective tissue

Cornea
Cornea

• Three keys to understanding grafting
  – The fate of transplanted nucleated cells
  – Endothelial function and decline
  – Immunologic nature of the cornea

Fate of Nucleated Cells

• Human Lymphocyte Antigens (HLA) are expressed on the surface of nucleated cells and identify the cell as part of the organism, or foreign
• HLA markers are the ONLY component of a transplant that can be identified as foreign by host immunity
• HLA markers only reside on nucleated cells, not connective tissue

Fate of transplanted cells

• Nucleated Cells are the only targets of rejection in grafted tissue.
  – Epithelium – transient, replaced in first month
  – Keratocytes – transient, unclear duration, likely somewhere between 6-60 months with central cells lasting the longest
  – Endothelium – permanent
Cornea: endothelial function

- The endothelium’s primary function is to maintain dehydration of cornea relative to aqueous by way of NA/K+ ATPase pump function.

- Born with a gross excess of cells, near 4,000 c/mm².
- This number declines throughout life at a rate of 0.6% per year via apoptosis.
- Sufficient until level drops to around 700c/mm².
- At this point, corneal edema occurs – called endothelial decompensation.
- Endothelial decompensation occurs at an accelerated rate in grafted eyes.
- This is accelerated further when certain types of rejection takes place.

Cornea: Immunology

- Corneal tissue rejects at much lower rates than other tissue.
  - Cornea tissue is not routinely HLA matched from donor to host.
  - CDS showed PKs have an 85% 5 year success rate.
  - Skin transplants that are not HLA matched will reject at near 100%.
Cornea: immunology
-- Considered immune privileged
  • Weak expression of Major Histocompatibility (MHC) antigens
  • Lack of both afferent (lymphatic) and efferent (vascular) arms of immune system
  • Lack of substantial load of native immune cells/APC
  • Presence of the Fas Ligand
  • Beneficiary of the Anterior Chamber Associated Immune Deviation (ACAID)

Corneal Immune Privilege: Weakly antigenic tissue
-- Donor epithelial Cells are rapidly turned over into host cells
-- Keratocytes are sparsely distributed and eventually replaced
-- Only endothelial donor antigen is permanent

Corneal Immune Privilege: Lack of afferent and efferent arms of immune system
-- Absence of lymphatics and blood from normal cornea minimizes both exposure of graft antigen and delivery of activated immune cells to the graft
Corneal Immune Privilege: Few native APCs in the graft

– Donor Derived APCs (passenger APCs) are a major cause of graft rejection in organ transplants and mediate what is known as the “direct route” or rejection
– In corneal grafts, with both few passenger APCs and no lymphatic system for delivery to central immune organs, these do not cause rejection

Fas Ligand

• A transmembrane protein expressed on certain immune privileged cells which induce apoptosis of infiltrating lymphocytes
• Highly antigenic mouse corneas genetically modified to not express the Fas Ligand reject 100% of the time compared to 65% of those that express it

Corneal Immune Privilege: The Anterior Chamber Associated Immune Deviation

– A paradoxical down-regulation of immune activity in response to an antigen derived from the anterior chamber
– Leads to creation of a species of immune inhibitory T-Cells
– Only alters delayed hypersensitivity pathway, the same pathway which mediates corneal graft rejection
Cornea Immune Privilege

• How much inflammation will immune privilege tolerate?
• What happens to immune privilege and vascular equilibrium when you put a suture through the cornea?
• What about when you put a contact lens on it?
• What about when you have chronic dry eye?
• What about when you are using glaucoma medications?
• What about when you have glaucoma surgery?
• What about when you have infection to the graft?

Immune privilege is relative!

Keratoplasty types

Background: 3 primary graft types

• **Full Thickness**: Penetrating Keratoplasty (PK) – full thickness transplant containing all layers of the cornea
• **Anterior Lamellar**: Deep Anterior Lamellar Keratoplasty (DALK) – all tissue anterior to Descemet’s membrane transplanted – useful for scars and ectasia
• **Posterior Lamellar**: Endothelial Keratoplasties – family of posterior lamellar grafts useful for endothelial decompensation
Penetrating Keratoplasty (PK)

One surgery can be applied to all layers
Relative ease of procedure
Historic record
Best overall survival rate!?
• **Weakness**  
  • Open Sky procedure means susceptible to expulsive hemorrhage  
  • Susceptible to all rejection types  
  • Dependence of chronic steroid use  
  • Acceleration of endothelial decompensation relative to physiologic levels  
  • Potential for wound rupture  
  • Astigmatism/rgp dependence  
  • Prolonged visual recovery

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**PK: life expectancy of graft**

• Life expectancy is roughly 15-25 years as, on average, ECD is reduced by about 30% by the surgery itself and decompensation proceeds at a more rapid pace than normal corneas\(^2,10\)  
• After 15 years average ECD is 800 c/mm\(^2\) of graft

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**DMEK complete air fill**

• Descemet Membrane Endothelial Keratoplasty (DMEK)
PLKs

• **Strengths**
  - Refractivity close to neutral – no dependence on rgps
  - Best corrected acuity as good or better than PK for similar indications
  - Very rapid recovery relative to PK
  - Quick recovery and strong visual outcome allows for earlier intervention
  - Rejection episodes less severe and less likely to lead to failure
  - More stable globe than with PK

• **Weaknesses**
  - Relative complexity
    - Steep learning curve
    - Prone to early dislocation and failure
  - Greater failure rate than with PK
  - Loss of donor tissue during graft preparation
    - DMEK – 8%
PLK life expectancy

- Endothelial decompensation to the graft caused by the surgery is variable with the graft type, insertion technique and incision size
- DSAEK - 28-32% ECD loss at 6 mos
- DMEK – 34-42% ECD loss at 6 mos4
- Acceleration of endothelial decompensation is about equal to that of PK
- In an uneventful recovery, life span will likely mirror that of PK

Rho-associated Kinase inhibitor drops (ROCK)

- Ultimately, this discussion on posterior lamellar keratoplasty may be unnecessary as a medical treatment for endothelial decompensation is being investigated
- ROCK drops paired with trans-corneal freezing may promote endothelial proliferation and migration, and thus clearing of corneal edema

Deep Anterior Lamellar Keratoplasty (DALK)
Deep Anterior Lamellar Keratoplasty

- Anterior cornea is transplanted with goal of leaving only host descemet's membrane and endothelium
- Useful in cases of anterior stromal disease: kerato-ectasias, corneal scarring

Graft Review:
DALK Big Bubble Technique

DALK

- **Strengths**
  - No endothelium transplanted so risk of rejection leading to failure is low/absent
  - No/minimal effect on endothelial decompensation
  - Less long term dependence on steroids
  - Stronger tectonically than PK
DALK

- Weakness
- Astigmatism/rgp dependence
- Prolonged visual recovery
- Surgical complexity often requires conversion to PK
- Greater failure rate than PK

DALK life expectancy

- In an uneventful recovery, should persist indefinitely

Quick Case: the benefit of DALK

- 69 YOM
- Ohx:
  - 2000 PK for corneal scar
  - Rejected 5 times in 4 years. Graft failed in 2004
  - Re-PK, 1 rejection episode in first year
  - Ongoing severe glaucoma developed in interim
    - 2013 DSAEK to PK
    - Process and postop resulted in increased IOP and exacerbated glaucoma
  - Currently BSVA to 20/150
Quick Case Take home

- 100% of these problems would have been avoided with DALK

Post operative Responsibilities of the OD

1: Visual Rehabilitation

Visual Rehabilitation

- This process takes 2-4 years for PK and DALK
- It occurs much more rapidly for DSAEK and DMEK
Visual Rehabilitation

Why do DALK and PK stabilize so slowly?

Sutures

- Sutures are necessary for DALK and PK graft fixation
- Though not the only source of astigmatism, sutures are a key contributor to refractive irregularity and slow visual recovery with these transplants
- There are two common types of sutures used in transplantation:
  - Running Suture
  - Interrupted Suture
- The specific type used has an impact on refractive outcome and timing for suture removal

Interrupted Sutures

- Interrupted Sutures are the standard suture for keratoplasty
- Involves placement of multiple single sutures, typically 16
- More time consuming for surgeon
- If a suture breaks early, it's usually not a problem (in contrast to running sutures)
Running Suture

- Typically a 24 bite single continuous suture running the circumference of the graft
- Quicker to place intraoperatively, though technically less forgiving
- If the suture breaks early, patient needs to go back for re-suturing of the graft

Manipulation of Astigmatism with sutures

- Both Interrupted and running sutures may be manipulated during the post operative course to modify astigmatism
- This manipulation is quite different among the two types

Manipulation of suture induced astigmatism: Selective suture removal

- With interrupted sutures removal of sutures in the steep meridian will result in a flattening of that meridian.
- Several diopters of astigmatism can be treated in this way
- However the effect is neither controllable or predictable
Astigmatism: Selective suture removal

Manipulation of suture induced astigmatism: Running Suture adjustment

Running Suture Adjustment

PENETRATING
KERATOPLASTY
SUTURE ADJUSTMENT

James McNeill, MD
So what’s better?

• Both interrupted suture removal and running suture adjustment can be effective means of astigmatism titration with running sutures offering perhaps more precision but at the cost of complexity and risk
• Each suture presents its own strengths and weaknesses
• Ultimately, suture type used boils down to surgical prerogative

Individual suture removal
(with interrupted sutures)

• Sutures should be buried, they will stain with fluorescein only when exposed or loose
  – ...what do you do with a loose interrupted suture?
• Sutures are antigenic. They promote vascularization and can lead to infiltration of the suture bite, which can then stimulate rejection
• In eyes where sutures are acting as an antigen, they should be removed as soon as possible

What about total suture removal?

• In a quiet eye, the sutures may be left in place indefinitely if the patient is happy with refraction – understand removing sutures has potential to dramatically alter refraction
• Average time to complete suture removal is somewhere between 18-36 months with a running suture
PK and DALK Astigmatism – surgical remediation

- Post suture removal, high amounts of remaining toricity may be treated surgically
- Relaxing incisions – LRIs on the graft
- PRK – PRK is better able to minimize irregular astigmatism than LASIK and with use of MMC is no longer as prone to causing haze

Astigmatism in PK and DALK

- Contact lens use is required in 50% of post PK eyes
- Up to 30% of patients post PK or DALK have suboptimal corrected vision secondary to astigmatism
- Unresolved pathologic astigmatism is a cause of graft failure

Visual Recovery: Posterior Transplants

- DSAEK tends to induce somewhere between 1-2 diopters of hyperopia. May be a consideration when coming up with refractive planning for patients getting ready to undergo cataract extraction who may also need DSAEK at the same time or shortly in the future
- DMEK creates essentially no refractive shift
- Refraction usually stable in these cases around 6 months with DMEK being more rapid to achieve stability
Post operative Responsibilities of the OD

1: Visual Rehabilitation
2: Graft Preservation

Post operative goal #2: Graft Preservation:

- Preservation of the transplant – is really the primary goal in the post operative period.
- We are trying to prevent “graft failure”
- Graft Failure is defined as a non-functional transplant for whatever reason.
- Failure may be subdivided into early and late failure

Graft Preservation

- **Early Failure**: a non-clearing full thickness graft or non-adhering endothelial graft
  - May be subdivided further into primary and iatrogenic failure
    - **Primary failure**: indicates early failure as a result of insufficient graft function
    - **Iatrogenic failure**: indicates surgical handling caused failure of graft – not common with PK, very common with EKs in inexperienced (~20%) surgeons progressively less common as surgeon hones technique (1-5%)”

- **NOT REJECTION**
Quick Case
1 day post DSAEK. HM vision
- Refloated that day with a large air bubble
- Next day VA 20/200 (due to air bubble interference, 1 week later VA 20/30)

Normal Day 1 DSAEK/Early Dislocation

Early vs Late Failure
- Early failure is a surgery dependent feature. As ODs we have no ability to prevent it and no ability to treat it. Late failure on the other hand...
Late Failure

- Late failure of a graft refers to a non-functional graft occurring after the immediate postoperative period. There can be a number of causes for failure.
- Sources of failure vary with graft type.
  - Keratoconus
    - Mean graft survival ~14.38 years
  - HSV scar
    - Mean survival 11.14 years

Late Failure may be caused by pathology or simply the graft endothelium failing.

- Late Failure causes
  - Endothelial Failure
  - Infection
  - Neurotrophy/ocular surface disease
  - Pathologic astigmatism
  - Wound Rupture
  - Glaucoma***
  - Rejection
  - Vascularization

Late Graft Failure: Endothelial Failure (PK and Posterior Grafts)

- ALL PKs and Posterior grafts will fail
- Expected life span of specific graft types prior to endothelial failure
  - PK – 20 years - 15 years post op average ECD is 800 c/mm2 of graft
  - DALK – endothelial decompensation occurs at physiologic levels
  - PLK – unclear, probably inline with PK based on early ECD loss rates at 5 years
- DSAEK - 28-32% ECD loss at 6 mos
- DMEK – 34-42% ECD loss at 6 mos***
Going…going…Gone. Failed PLK

The good news about endothelial failure?
• We can now treat grafts with failed endothelium with the posterior lamellar transplants

Late Graft Failure: Infection
• All standard immune modulators used to promote graft survival increase risk of infection
• High dependence on contact lenses for PK and DALK
• Infection is often with atypical organisms
• High rate of ocular surface disease with PK and DALK
• Herpetic indications have a 25% risk of reactivation within the first year of transplanting
Late Graft Failure: Neurotrophy/Ocular Surface Disease (PK and DALK)

- Severe Ocular Surface Disease
  - As corneal nerve plexus are severed by these surgeries, PK/DALK inherently create a more neurotrophic cornea
  - Many indications for grafting also cause neurotrophy
  - Epithelial sloughing occurs regularly with PK in the early phase of recovery
  - An epithelial defect in the setting of a neurotrophic eye is a potentially dangerous situation

Role of Amniotic Membrane

- Represents an “inflammation sink” with known antiseptic properties which also provides an artificial basement membrane for epithelial scaffolding
- Useful in cases of inflamed/neurotrophic cornea which are frequently encountered with PK and DALK grafts
- Not strong enough or deep enough of an effect to treat rejection episodes, but likely useful in managing acute surface disease which can lead to graft infiltration and vascularization

Quick case
Quick Case

Preservation of the graft: Globe stability (PK primarily)

- Incision size is on average 24 mm. (7.5mm diameter X pi)
- Rupture incidence reported between 2.5-5.8%
- Incidence of DALK rupture is ~0.5%
- Highlights the importance of protective eye wear for your transplant patients

Preservation of the graft: Glaucoma (all grafts)

- The first or second leading cause of vision loss after a PK
- Rate of glaucoma after PK is 18-35%
- Patients with pre-existing glaucoma have a shorter graft survival 71% at 3 years compared to 89%
- In addition to causing nerve damage, poorly controlled IOP also damages endothelium accelerating decompensation of the graft
Preservation of the graft: Glaucoma

- Treatment is complicated by need for continued immune suppression with corticosteroids
- Surgical intervention occurs more frequently in this setting, but surgery dramatically increases risk for failure of the graft

Preservation of the graft: Glaucoma post transplant

- Graft Failure after:
  - Tube – up to 51%
  - Trab with MMC – 12-16%
  - Cyclodestruction – 17-43%

- In a nutshell: Glaucoma damages the graft and causes irreparable vision loss through nerve damage, but its treatment damages the graft

Preservation of the graft: Glaucoma

- The good news:
  - Lamellar transplants have a much lower tendency to induce glaucoma, both from the surgery itself, and as a result of reduced dependence on corticosteroids
Graft Failure: Rejection (all grafts)

• Terminology
  – Rejection and failure are two different things
  – The term rejection, which may be a source of failure, indicates the body has mounted an immune response against the graft. What evidence of this is required clinically?
• WHITE CELLS WITHIN THE GRAFT

Late Failure: Rejection

• Rejection – CD4+ T cell mediated, attack mounted by the host against the foreign graft.
• Rejection may lead to graft failure through collapse of endothelial pump, or occasionally, scarring
• Rejection is the most common cause of graft failure among PKs, represents a significantly less common source of graft failure among lamellar grafts

Types of Rejection

– Epithelial Rejection
– Stromal Rejection
– Endothelial Rejection
Types of Rejection

- Epithelial rejection – Body attacking epithelial cells
- Manifests as a grey slightly elevated epithelial zone
- Does not cause failure
- Limbus is the stem cell region for epithelium and is not transplanted (with exception of KLAU) – therefore, host epithelial cells will eventually replace donor regardless of rejection

Types of rejection

- Stromal rejection – Body attacking keratocytes
- Anterior stromal nummular zones of infiltration
- Does not cause failure unless significant scarring results

Types of Rejection

- Endothelial rejection – Body attacking endothelial cells
- Manifests as keratic precipitates. May be paired with stromal edema
- Represents immune response against endothelium which persist indefinitely
- May cause failure via endothelial collapse
- Shortens the life expectancy of the graft even when effectively treated
Quick case: Non-Acute impact of endothelial rejection

Graft is 5 years old with one documented case of rejection within the first year of transplantation.

Risk of Rejection is relative to graft type

- Penetrating Keratoplasty – 18%
- DALK – 4% - none progressing to failure
- PLK
  - DSAEK – 12%
  - DMEK – 1%
- Low risk of rejection with lamellar grafts does not correlate with no risk of rejection

Quick Case

- Rejection tends to be less severe and leads to failure less commonly with lamellar grafts
- Rejection of PLK
**Treatment/prevention of rejection**

- Primary treatment of rejection is prevention
  - Options
    - Blanket reduction in immune response
      - Corticosteroids, topical or oral
    - Specific reduction in T cell activity
      - Topical Cyclosporin A 1-2% or tacrolimus
        - Has to be combined with corticosteroid for full effect
      - Oral immune modulators – mycophenolate (Cellcept) tacrolimus (Prograf)

**Typical taper strategies in uneventful recoveries**

- PK – typically starts with prednisolone acetate dosed qid-q2h. This is then tapered by one drop every 3-6 months, eventually transitioning to lotoprednol with stopping considered in the 18-24 month range
- DALK – PA1% beginning at qid may be tapered over the first 6-12 months postoperatively
- DSAEK – PA1% qid for 3 months tid for 3 months, bid for 3 mos, qd for 6 months
- DMEK – similar taper to DALK

**When rejection attacks**

- When a rejection episode occurs, initial treatment is an immediate increase in topical steroid...don’t be shy with the dose
- Taper from here only after rejection episode clears
- Any rejection episode means the patient must always maintain some dose of steroid in the setting of a PLK or PK
- Over 50% of acute rejection episodes in a PK can treated effectively
Corticosteroid therapy is often ineffective in management of high risk grafts. Additionally:
- Corticosteroids create significant morbidity of their own
  - OHTN
  - Cataract formation
  - Increased risk of infection or reactivation of herpetic disease
- Oral Immune Modulators
  - Effective and spares OHTN and Cataract formation but this class has its own undesirable side effects. You know, like infection, cancer and death…

Rejection treatment. The bad news

Treatment/prevention of rejection
- Are there ways to reestablish immune privilege in corneas that are losing/have lost it?
  - Reduction of corneal APC?
  - Modification of ACAID?
  - Increased expression of Fas Ligand?
  - Modification of expression of MHC?
  - Reduction of vascularization/lymphatics?

Role of Vasculature in Rejection (for full thickness grafts)
- Vascularization ≠ rejection
- It is, however, the primary risk factor for rejection.
- Therefore, reducing vasculature should have a positive impact on graft survival:
  - 15-20% risk of rejection in avascular grafts
  - Over 50% risk of rejection in significantly vascularized grafts
What about Lymphatics?

- Lymphatics may be at least equally implicated in corneal graft rejection
  - Studies have shown presentation of graft antigen will not stimulate rejection up to a certain level presented. When that level is exceeded, rejection takes place
  - Lymphatic channels are the feature that result in greater antigen presentation
- Removal of regional lymph nodes can induce permanent survival of even high risk grafts

Relationship between corneal blood vessels and lymphatics

- Neovascularization and lymphangiogenesis are tied processes in the cornea.
- As lymph channels are clinically (and nearly histologically) invisible, the level of lymphatics can be roughly equated to the level of blood vessels

Role of Vascularization in Corneal Transplant survival

- Reducing vasculature/lymphatics is possible and has been studied with currently available anti-VEGF medications
  - bevacizumab (Avastin)
  - aflibercept (Eylea)
- Both of which inhibit corneal angiogenesis and lymphangiogenesis
Dosing bevacizumab for graft related Neovascularization

- Has been used topically
- Has been used subconjunctivally
- Has been used intrastromally

What do we know about anti-VEGF treatment from retinal utilization

- Mechanism of action is on newly developed/developing vasculature
- Duration of intravitreal action with bevacizumab is about 4-6 weeks, slightly longer with aflibercept

**HOWEVER...**

- What we know about anti-VEGF therapy from use with retinal vascular disease cannot be simply applied to the cornea
  - Pharmacokinetics are undefined with the various techniques – undefined treatment dose effect and need for re-treatment
  - Sporadic reports of success in treating established vessels
  - Sporadic reports of failure with early, presumably susceptible vessels
  - It remains, as with retinal use, a “non-Miracle” drug, but has intriguing applications

Quick Case
Does this matter for lamellar grafts?

- Case reports of graft failure due to vascularization are published for both PLKs and DALK – while rejection may not occur in these cases, the vascularization itself may lead to opacification of the graft and failure.
Avastin...good for everything?

- Intrastromal Avastin dosed as adjunctive therapy for interstitial herpetic keratitis.

The last frontier: “Priming” eyes prior to (or at the time of) transplant

- Timing of treatment could play a large role with suppression of VEGF-A early in recovery possibly being more effective than late suppression.
  - Early suppression allows for establishment of ACAID derived tolerance.
  - In the presence of early lymph channels ACAID is not developed.
  - May be paired with large vessel cauterization.

Pretreatment of high risk cornea should reduce risk of rejection.
Conclusion

• Due to the privileged status of the cornea, corneal transplants have the best success rate in of all transplants
• Despite this, many postoperative challenges remain which can threaten the graft
• Modulation of the immune response is mandatory for graft survival
  – Established therapies are effective for most transplants but carry well known potential for complication
  – Newer therapies are showing promise both with safety and efficacy for higher risk grafts, though treatment protocol remains undefined
• It's up to us as the patient’s primary eye care provider to ensure both survival and optical function of their transplant

Questions?

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