


Don't wait... REGENERATE!!!
How to use regenerative tools in eyecare

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Disclosures

- Consultant, Medical Advisor, Speaker, or Paid Investigator:
 - BioTissue/TissueTech, Domeq, EyeVance Pharmaceuticals, Glaukos/Avedro, Horizon Pharmaceuticals, Hovione, Kala Pharmaceuticals, NicOx, NuSight Medical, Ocular Therapeutix, Science Based Health, Sight Sciences, Syntesis, TearReStore, Tear Solutions
- Editorial Board/Reviewer:
 - Modern Optometry, Optometry Times, Journal of Dry Eye Disease
- Co-Administrator: OSDocs (facebook)



• This lecture may discuss off-label uses of some medications or devices



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What is "Regenerative Medicine"?

- Utilizing biologic tissues and extracts to repair wounds and restore tissues and organs to their normal healthy state
- Idea of "Stem cell therapy" - healing of organs and tissues goes back to Greek philosophy - *Promethius*

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True "stem cell therapy"

- Consists of utilizing cells/tissues with the following properties:
 - 1) Self-renewal (proliferate)
 - 2) Clonality (arising from a single cell)
 - 3) Potency (ability to differentiate into different cell types)

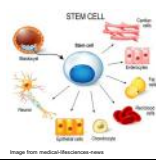



Image from medicalillustrations.com

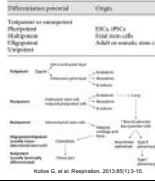


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Hierarchy of stem cells

- Totipotent – derived from blastocoele
- Pluripotent – can proliferate into multiple cell lines (from blastocyst)
- Oligopotent – derived from adult tissue

Differentiation potential	Origin
Totipotent or multipotent pluripotent	ESC, iPSCs
Pluripotent	Embryonic stem cells
Oligopotent	Adult or mesenchymal stem cells
Unipotent	




Kalish G, et al. Regeneration. 2013;85(1):3-10.



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Are we using stem cell therapy?

- Technically, no.... BUT
- We are harnessing aspects of the environment created within the womb, which may occur prior to the development of mature biologic healing systems



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How do RM therapies differ from traditional pharmaceutical / surgical interventions?

- Pharmaceutical tools typically target one aspect of wound healing:
 - Calcineurin inhibitors – T-cell recruitment / activation
 - Steroids – inflammatory cascade
 - Surgery – mechanical / architectural changes
- RM techniques attempt to restore physiology via restoring anatomy
 - Affects multiple aspects of wound healing



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A closer look at the tools and what sets them apart


Eye Care Options in Regenerative Medicine



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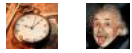
Available regenerative therapies in ophthalmics

- Amniotic membrane
 - Cryopreserved vs. dehydrated
- Amniotic membrane extract drops
- Amniotic fluid drops
- Autologous serum
- Platelet-enriched plasma drops
- Umbilical cord serum drops



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Historical Use of Amnion



- 1912 – First utilization of amnion in general surgery (skin grafts)
- 1940 – First ophthalmic use (conjunctival defects)
- 1946 – Treatment of caustic burns of the eye
- 1995 – Human AM used to minimize neovascularization in rabbit corneas
- 1996 – Used in treatment of OCP and SJ syndrome
- 1998 – Recurrent pterygia associated with symblepharon
- 2007 – First self-retaining amnion tissue product receives FDA approval

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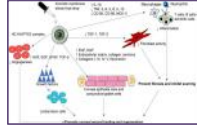
Promotion of epithelialization

- Basement membrane of AM similar to BM of corneal epithelium
 - Facilitates epithelial migration
 - Regulates ECM deposition
 - Reinforces basal cell adhesion
 - Promotes epithelial cell progenitor differentiation
 - Prevention of apoptosis

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Role of biologics


- Exert the following:
 - Anti-inflammatory
 - Decreases expression of
 - CD80, CD86, MHC-II antigens
 - Downregulation of
 - TGF- β , TNF- α , IL-6, IL-12
 - Anti-scarring
 - Downregulation of TGF-1 & TGF-2



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Biologic components in AMT

- Epidermal growth factor (EGF)
- Hepatocyte growth factor (HGF)
- Platelet-derived growth factor (PDGF)
- Basic fibroblast growth factor (bFGF)
- Transforming growth factor (TGF)
- Fibronectin
- HCHA-PTX3*



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Growth factors important in RM

- Epidermal growth factor (EGF)
 - May be one of primary biologics leading to corneal wound repair
 - Nakamura, et al – prevented ED from healing by installing EGF-antagonist
 - Particularly sensitive to processing
 - Stadhoff et al. Curr Eye Res 2017
- Nerve growth factor (NGF)
- Hepatocyte growth factor (HGF)
- Basic fibroblast growth factor (bFGF)

Biologic/Protein	Function	Effect
EGF/FGF	Epithelial repair	Accelerates
HGF	Wound healing	Accelerates
PDGF	Cell proliferation	Accelerates
bFGF	Cell proliferation	Accelerates
TGF- β	ECM production	Accelerates
FGF-2	Cell proliferation	Accelerates
IGF-1	Cell proliferation	Accelerates
IGF-2	Cell proliferation	Accelerates
IGFBP-3	IGF-1/2 inhibition	Retards
IGFBP-4	IGF-1/2 inhibition	Retards
IGFBP-5	IGF-1/2 inhibition	Retards
IGFBP-6	IGF-1/2 inhibition	Retards
IGFBP-7	IGF-1/2 inhibition	Retards
IGFBP-8	IGF-1/2 inhibition	Retards
IGFBP-9	IGF-1/2 inhibition	Retards
IGFBP-10	IGF-1/2 inhibition	Retards
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IGFBP-98	IGF-1/2 inhibition	Retards
IGFBP-99	IGF-1/2 inhibition	Retards
IGFBP-100	IGF-1/2 inhibition	Retards

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Tissue Prep: Dehydrated v. Cryopreserved

Dehydration (PURION™ method)	Cryopreservation
• Amnion/chorion separated	• PBS rinse
• Tissues are rinsed	• Chorion/amnion separated by blunt dissection
• Epithelium removed	• PBS rinse
• Tissue dehydrated (temp?)	• Cut to 6cm x 6cm
• Gamma-radiation applied for sterilization	• Stored in 1:1 glycerol/Dulbecco's Modified Eagle medium
	• Frozen at -80C

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Other functions of AMT

- Drug delivery
 - Hazarika M, et al. Indian J Ophth 2021 May;69(5):1068-1072.
 - 1% voriconazole impregnated AM buttons;
 - Noted drug release up to 5 weeks
 - May be helpful in fungal keratitis
 - Moxifloxacin (in vitro) – Yelchuri ML, et al. Cornea 2017 May;36(5):594-599.
 - Studied release kinetics in moxi-impregnated AMT
 - No significant release between soak times
 - Sustained release up to 7 weeks

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Harvesting of various RM products

- AMT, AME, UCS, HAF – at time of Cesiarian section
- Blood serum, PRP - donation via phlebotomy
- May be varying concentrations of biologics

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Processing of AMT

Cryopreserved	Dehydrated (PURION™ method)
• PBS rinse	• Amnion/chorion separated
• Chorion/amnion separated by blunt dissection	• Tissues are rinsed
• PBS rinse	• Epithelium removed
• Cut to 6cm x 6cm	• Tissue dehydrated (temp?)
• Stored in 1:1 glycerol/Dulbecco's Modified Eagle medium	• Gamma-radiation applied for sterilization
• Frozen at -80C	

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Processing of AME and AMEED

- Inconsistent; process varies (Baradaran-Rafii, Ocul Surf 2018)
 - Wash with 5% penicillin & streptomycin
 - Cuts into small pieces via scalpel
 - Submerge into liquid nitrogen
 - Mixture is homogenized, centrifuged, and supernatant is collected
 - Centrifuged again, sterilized via filtration (0.25mm)
- Other processes involve pulverization, micronization, morselization of CAM or DAM; this affects concentration of extracts

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Processing of AFED

- Harvested at time of Cesarean section
- From proteomic perspective, second trimester most beneficial with highest concentration of growth factors and organic salts/proteins¹

1) Shaw SMS, et al. Taiwan J Obstet Gynecol. 2017 Dec;56(6):770-774.

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Human Amniotic Fluid

Topical amniotic fluid: a potential new alternative for ocular surface diseases

- Amniotic fluid drops
 - Harvested, filtered and UV sterilized
 - 98-99% water, with organic & inorganic salts, desquamated fetal cells¹
 - Contents change according to when during pregnancy it is harvested
 - Organics:
 - EGF, IGF-1, VEGF, TGFβ1, TGFβ3, GDF-11²

1) Wang SH, et al. Clin Ophthalmol. 2016;10:1165.
2) GSE Science QJ, et al.

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HAF Eye Drops

Author	Year	Subject (n)	Time	Injury	Wk	Result
Lee & Kim	1998	Healthy (16)	1 wk	Epithelial abrasion	None	HEP repair improved
Hernandez et al	2006	Mice (20)	2 wk	Alkali burn	Wound drops, 10% cell growth	No side effects
Kemper et al	2008	In vivo	N/A	Cell growth in culture	None	Significant repair
Castro-Correa et al	2008	Rabbit (20) in vivo	72 hrs	Epithelial (burn)	Wound occlusion	No side effects
Quinto et al	2012	Mice (20)	2 wk	Dry Eye	Topical PDK, GDF, HGF, IGF-1	HEP repair, GDF side effects
Quinto et al	2016	Mice (40)	4 wk	Dry Eye	Topical PDK, GDF, HGF, IGF-1	HEP repair, PDK side effects
Chen et al	2019	20 mice, non-comparative	1 wk	Dry Eye	PDK, symptoms	HEP repair, GDF, HGF, IGF-1 improved

- Peer-reviewed literature support is not strong at present
- Anecdotally:
 - 1) Dry eye in elderly / chronically sick
 - 2) Neuropathic pain

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Utilization of Regenerative Therapies

How to use what on who and when

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How to choose what?

- Acute vs. Chronic problem?
- Tectonic repair?
- Mechanical protection?
- High levels of inflammation?
- Risk of scarring?
- Anatomical barriers?

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Utilization – Acute vs. Chronic

	Short term (1-3 mo)	Med term (3-6 mo)	Long term (6m - 6m)	Ongoing therapy
Autologous serum			X	X
PEP			X	X
UCS			X	?
AFED	X	X	X	?
AMT	X	?		

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Using RM tools

- May be helpful in any of the following:
 - Infectious keratitis (outside of sterilization)
 - Neutrotrophic keratitis
 - Persistent epithelial defects
 - Dry eye
 - Neuropathic corneal disease (i.e. neuralgia)

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Let's see how we do with these in the real world....

Case Examples

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Case 1: Alkalai burn

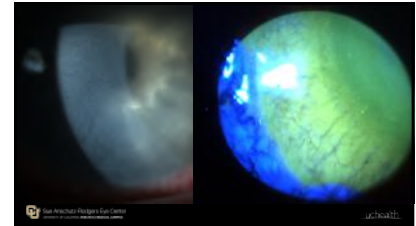
- 29 year-old male
- Working with cement mortar, mix splashed into right eye afternoon of day before
- Irrigated for between 2-4 hours initially (off and on), did not seek treatment right away
- Increasing pain and photophobia through next 24 hours
- Went to ER, diagnosed with corneal abrasion and referred to us
 - Irrigated for 10 minutes in ER, pH tested at neutral

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Case 1: Alkalai burn

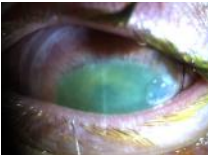
- VA(sc):
 - OD – 20/200; PH - NI
 - OS – 20/20
- Pupils: ERRL (-)APD
- EOMs: No restrictions
- Biomicroscope (right eye):
 - 2+ patchy erythema adjacent to lids
 - 2+ UL edema/erythema
 - 3+ conj hyperemia, no vascular blanching, 1-2+ mucus, 2+ chemosis; denuded limbal area 1-2mm past limbus 3:00 and 9:00 positions
 - 70% denuded cornea, 1+ ant stromal haze, no infiltrates
 - AC – Deep, tr-1+ cell
 - Irs normal

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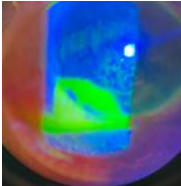
Case 1: Management



- Check pH (~7.0)
- Double amniotic membrane (PK+)
- Moxifloxacin QID
- Pred Acetate QID
- Vitamin C 2000mg/day
- NPAT as often as possible

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Case 1: 4-day F/U



- 4 days following injury:
 - Vasc 20/60-
 - 3.0 x 3.5mm epi defect at limbus; amnion nearly dissolved
 - Decreased Pred and Moxi to BID, cont Vit C 2G/day PO
 - Instructed to F/U 1 week

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Case 1: 11 day F/U

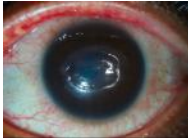


- VAsc 20/25+
- SLE:
 - No conj defect, no blanching
 - Cornea with spotty PEK, very light SE haze
- Taper medications, cont Vit C, aggressive lubrication

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Case 2: Persistent Epithelial Defect

- 37 year old male; +GVHD
- Ocular pain - worsening over 4-5 days along with worsening vision
- Presents with open epithelial defect OS
- Minimal conjunctival changes on exam



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Case 2: Management of PED

- CULTURE!
- Pred forte BID
- Moxifloxacin QID
- ProKera Plus
- ASEDS 25% QID OU
- 2-3 week F/U, 50% improvement, pending partial tars/amnion graft OS (culture neg)

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Case 3: Severe DES

- 84 year-old female
- Longstanding Sjogren's, RA, GERD
- Presents with long history of medications:
 - ASEDS, steroids, systemic immunomod
- Addition of AFD
- 6 week F/U – 50% improvement!



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Case 4: Neuropathic pain

- 45 year-old female
- Developed progressive discomfort and pain following LASIK surgery >10 years ago
- Uncontrolled pain on typical DE therapy
 - Burning, pain, tearing OS>>>OD
 - Borderline suicidal

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Case 4: Neuropathic pain

- TRIED:
 - Serum tears 25% x 1 year; NI
 - Restasis, couldn't tolerate
 - Xiidra x 1 week, couldn't tolerate
 - NPAT – no help
 - Punctal cautery

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Case 4: Neuropathic pain

- EXAM:
 - 9/9 OD and 10/10 OS incomplete blink
 - TBUT 7/5sec
 - LipiView: 100+nm OD and OS
 - Grade 1 LL OU with 8/15 clear mod
 - LASIK flap OU; no PEK OU
 - No LG stain OU

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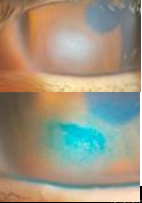
Case 4: Neuropathic pain

- Management:
 - ASEDs 50% QID OU
 - Similasan Dry Eye (TRPM8 stimulant)
 - Alpha Lipoic Acid 600mg BID PO
 - Conscious blinking
- 3 mo F/U: noting improvement
 - 90% in RE and 50% in LE

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Case 5: Neurotrophic/Interstitial keratitis

- 9 year-old female
- Hx of ALL+MLL
- +CMV retinitis, pneumonia now resolved
- Non-painful "white spot" OS
- VA = 20/40-



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Case 5: Management

- Moxifloxacin QID
- Lotemax BID
- Acyclovir 200mg/ml BID PO
- Regenereyes BID
- 2 mo F/U: No epi defect, decreased haze
- VA = 20/25

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Summary

- Regenerative therapies are extremely useful adjunct to traditional allopathic medicine
- Provide a means to potentially improve or restore normal ocular physiology
- Room for optometry to assist in developing information on efficacy and utilization
- Best when utilized early
- **DON'T WAIT.....REGENERATE!**

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