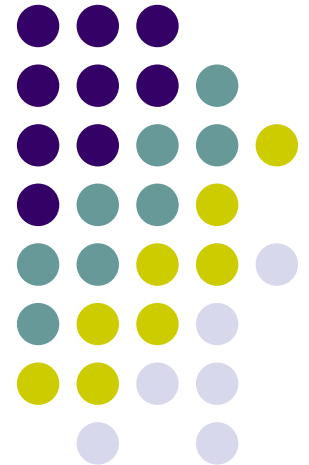
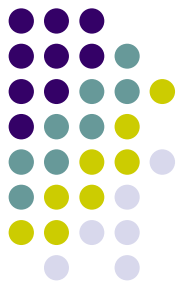


Normal Human Cell-Based In Vitro Epithelial Models for Drug Delivery and Toxicology Applications

Patrick J. Hayden, Ph.D.
MatTek Corporation



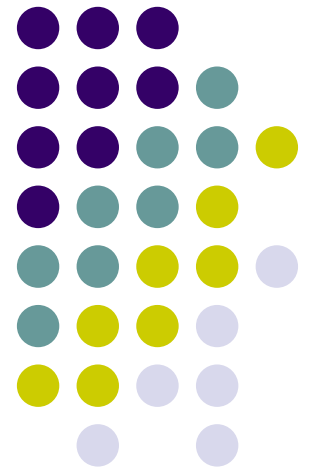


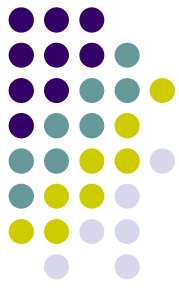
Overview

- **Basic Technique for Reconstituting 3-D Organotypic Epithelial Tissues**
 - Culture on Microporous Membranes
 - Medium Considerations
 - Extracellular Matrix Considerations
- **Survey of Normal Human Cell-Based Epithelial Models Produced at MatTek Corporation**
- **ADME/TOX Applications of an Organotypic Normal Human Airway Epithelial Model**
 - Nasal Drug Delivery

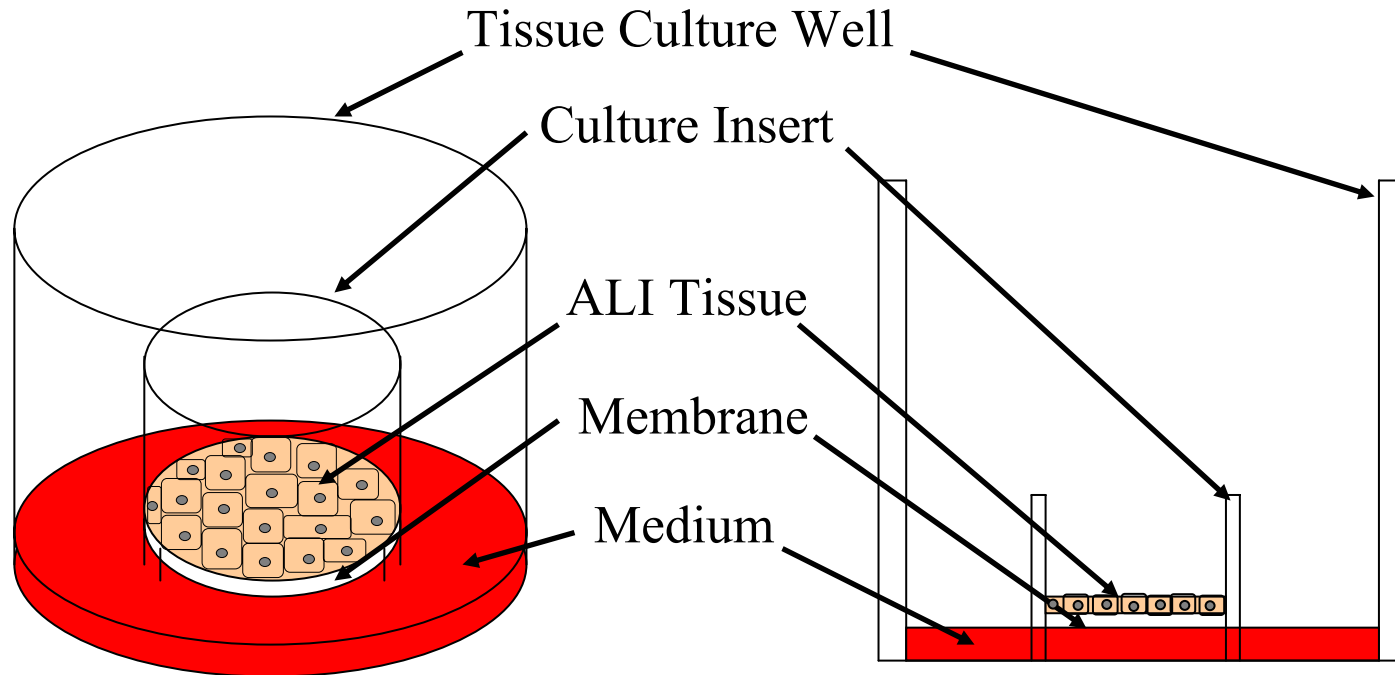
Section I

Basic Technique for Reconstituting 3-D Organotypic Epithelial Tissues

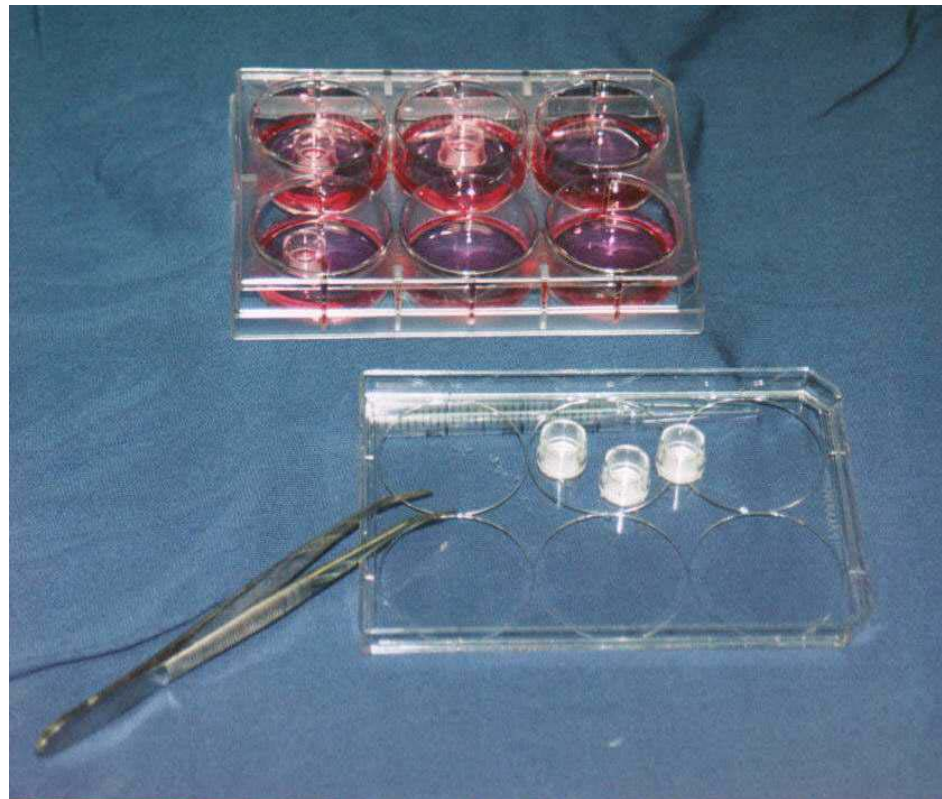




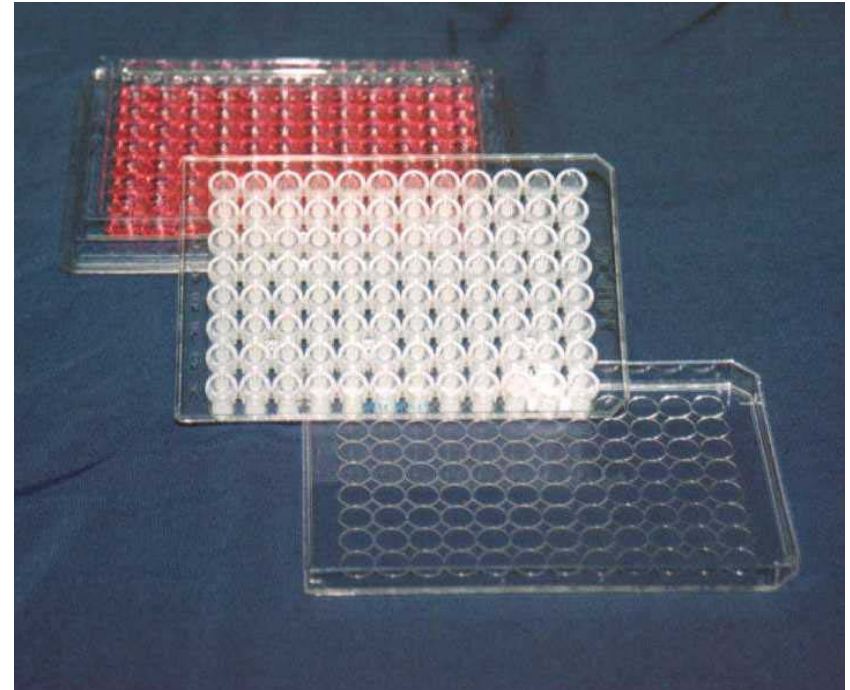
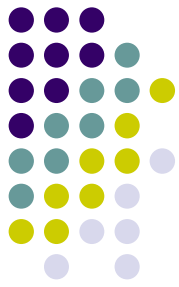
Culture on Microporous Membrane Inserts at the Air/Liquid Interface (ALI)



The Air/Liquid Interface (ALI) Tissue Culture Technique



High Throughput ALI Formats



Reconstituting 3-D Organotypic Epithelial Tissues from Normal Human Cells



- Primary Normal Human Cells Obtained from Donor Specimens
- Cell Types Separated and Expanded in Monolayer Culture to Provide Large Pools
 - Epithelial cells
 - Melanocytes
 - Fibroblasts
- Cells are Cultured on Microporous Membranes to Reconstitute Differentiated Tissues with Many Biochemical and Physical Characteristics of In Vivo Human Tissue

The Importance of the Differentiated State



- Physical Properties
 - Air/liquid Interface
 - Realistic Treatments
 - Barrier function
 - Stratum Corneum (Epidermis)
 - Tight Junctions (Tracheal/Bronchial)
 - Mucociliary Phenotype
- Biochemical/Molecular Properties
 - Gene and Protein Expression Patterns Change Dramatically with Differentiation- Critical Aspect for Obtaining Meaningful In Vivo-Like Results

Attaining a Differentiated Organotypic State



- Microporous Membrane
- Medium Considerations
 - Calcium Concentration
 - Retinoic Acid
 - Other specific growth factors and supplements
- Extracellular Matrix Considerations
 - Collagen I
 - Collagen IV
 - Fibronectin
 - Laminin

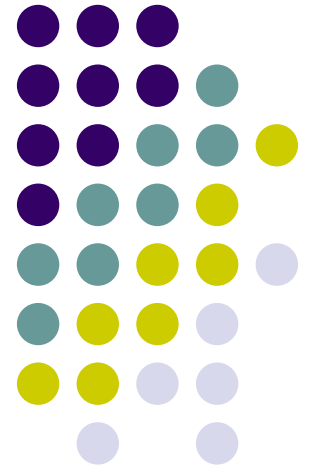


Summary of Section I

- Reconstitution of 3-D organotypic epithelial tissues involves:
 - culture of (normal, human) epithelial cells on microporous membranes
 - Appropriate medium and extracellular matrix to attain differentiated state
- 3-D Organotypic tissues provide:
 - In vivo-like physical and biochemical properties.
 - Realistic in vivo-like treatment protocols

Section II

Survey of Available Normal
Human Organotypic Epithelial
Models and Applications



In Vitro Human Epithelial Tissues Produced by MatTek Corporation



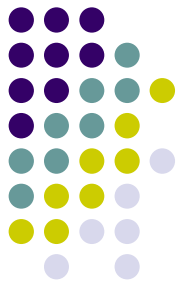
● “Simple” Tissues

- EpiDerm™
- EpiOcular™
- EpiAirway™
- EpiOral™
- EpiGingival™
- EpiVaginal™ (VEC)

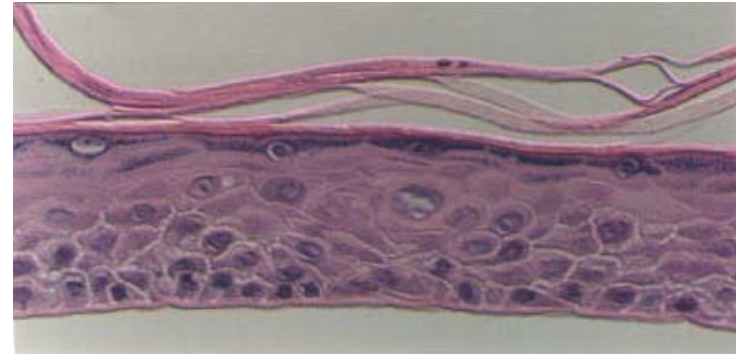
● Complex Tissues

- MelanoDerm™
 - Keratinocytes
 - Melanocytes
- EpiDermFT™
 - Keratinocytes
 - Fibroblasts
- EpiVaginal™ (VLC)
 - Ecto-Cervical
 - Langerhans Cells

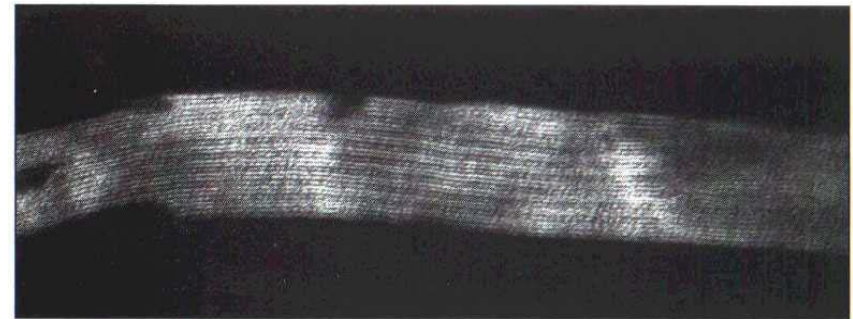
EpiDerm™ Human Skin Equivalent



- Normal Human Keratinocyte-Derived
- Stratified, Differentiated Morphology with Barrier Function
- Normal Ceramide and Lipid Profile
- Normal Ultrastructure of Intercellular Lamellar Lipid Sheets
- Defined Medium

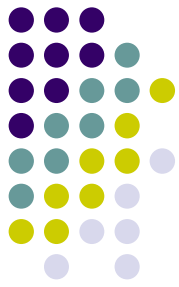


Histological Cross Section of EpiDerm 200. Mag = 440X

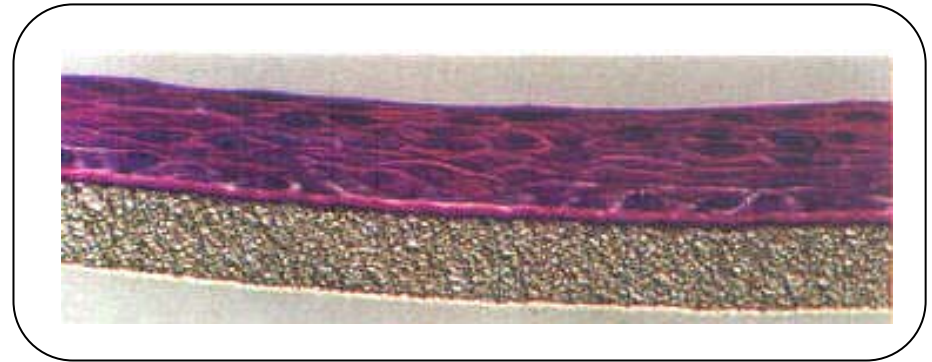


Transmission Electron Micrograph of Intercellular Lamellar Lipid Sheets: Broad-Narrow-Broad-Spacing (150K X)

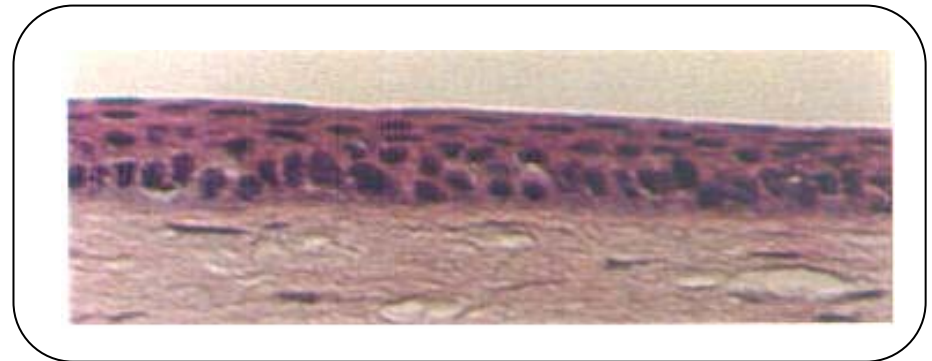
EpiOcular™ Human Corneal Epithelium Equivalent



- Normal Human Keratinocytes
 - Stratified Squamous Epithelium Similar to Cornea
- Good Correlation to In Vivo Draize Ocular Irritation Results

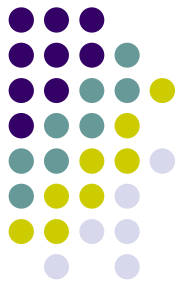


EpiOcular: Magnification 360x

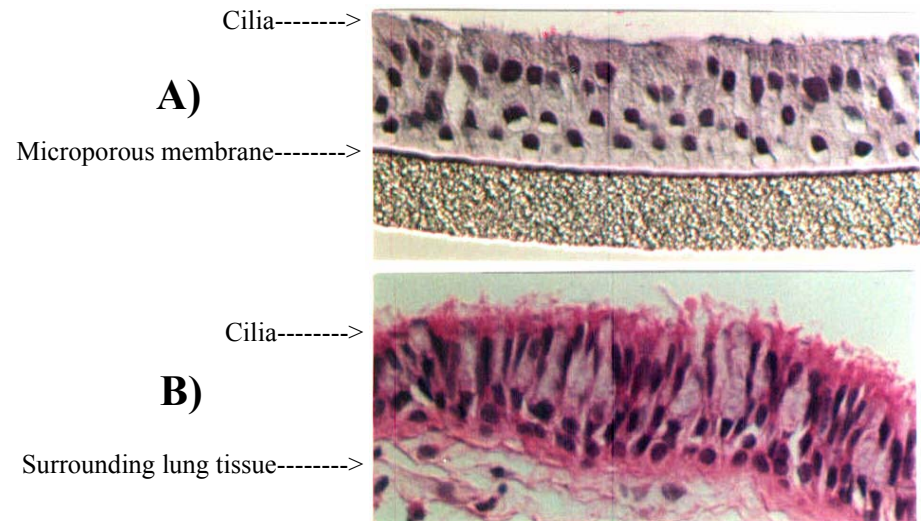


Rabbit Cornea: Magnification 360x

EpiAirway™ Human Tracheal/ Bronchial Epithelial Equivalent



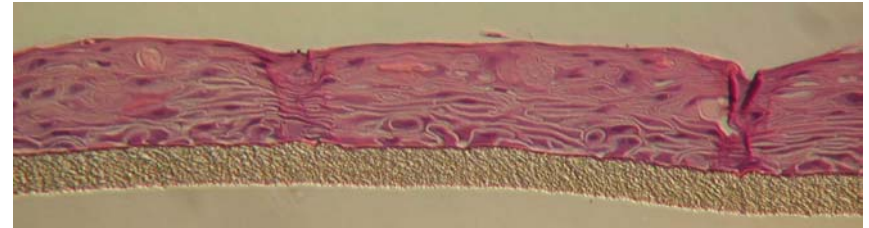
- Normal Human Tracheal/Bronchial Cells
- Stratified Tissue with Differentiated Mucociliary Phenotype
 - Actively Secrete Mucus
- Electrogenic
 - Tight Junctions
 - Ion Transport
- Utilized for Inhalation Toxicology, Drug Delivery, Gene Therapy and Basic Research Applications



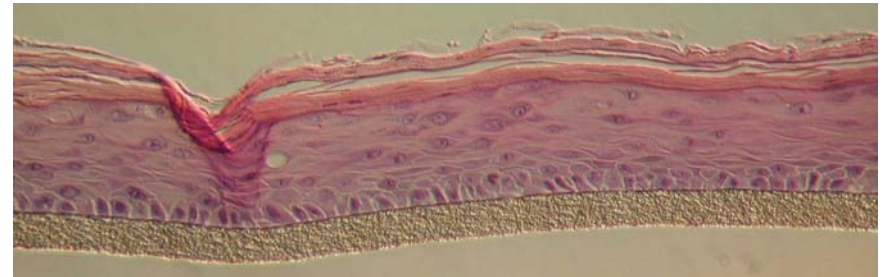
EpiOral™ and EpiGingival™: Human Buccal & Gingival Tissues



- Normal human gingival-keratinocytes derived
- Non-cornified (buccal) and cornified (gingival) phenotypes
- Applications
 - Drug delivery
 - Oral care products (irritation & efficacy)

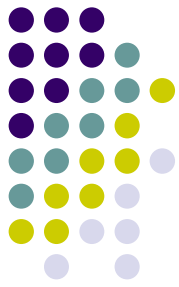


Non-cornified “buccal”
(ORL-100)

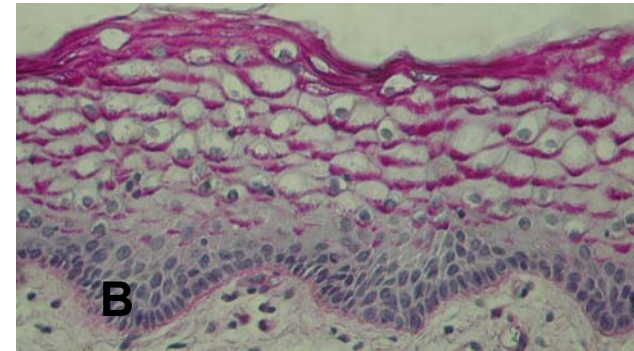
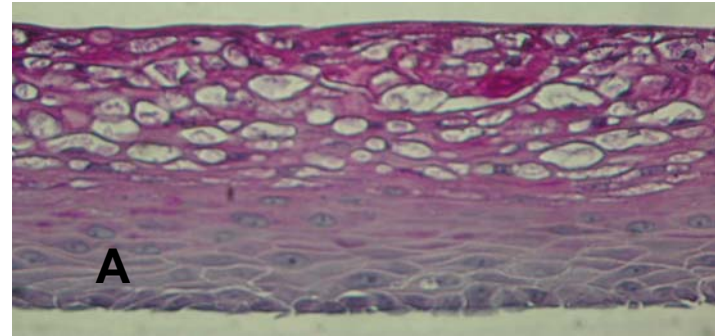


Cornified “gingival”
(GIN-100)

EpiVaginal™: Human Vaginal Ecto-Cervical Equivalent (VEC)

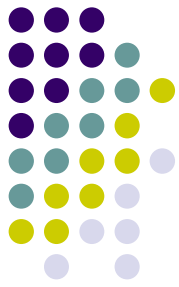


- Normal human vaginal-ectocervical (VEC) epithelial cells
- Multilayered, three-dimensional morphology
- Applications:
 - Toxicology
 - Drug Delivery
 - Basic Research
 - HIV infection

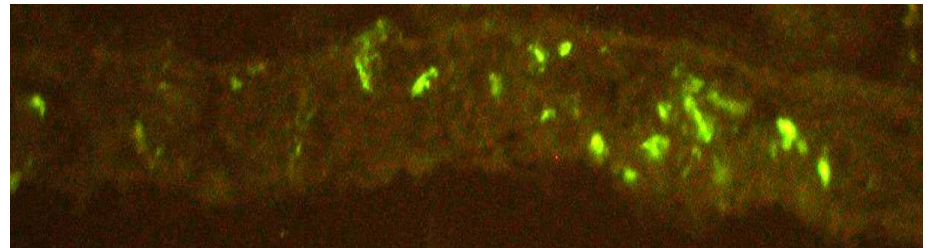


PAS reacted cross-sections of: **A) EpiVaginal** tissue and **B) excised human** vaginal tissue. Glycogen shows up reddish-pink. In both tissues, the intensity of glycogen staining increases as the apical surface is approached.

EpiVaginal-LC™: EpiVaginal with Langerhans Cells (VLC)



- Research on HIV infection and transmission
- Microbicide development
- Irritation and immunological studies

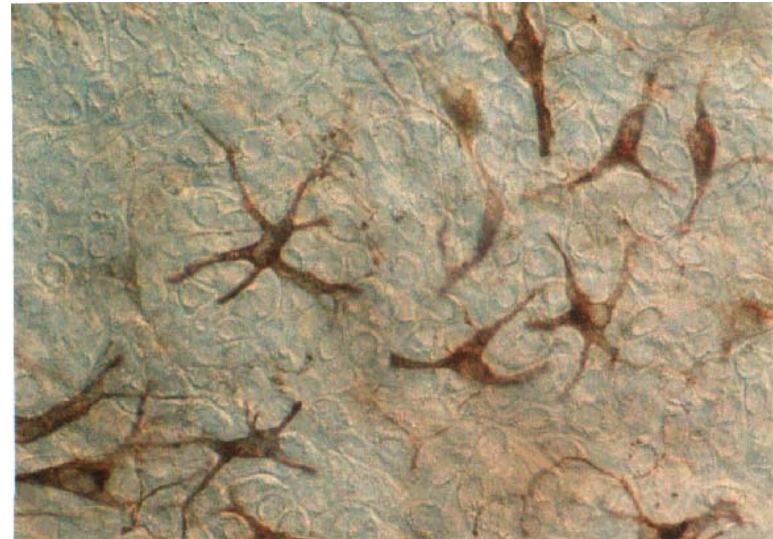


Immuno-staining of VLC-FT for HLA-DR

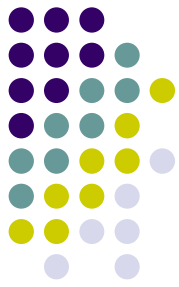
MelanoDerm™ Human Skin Equivalent



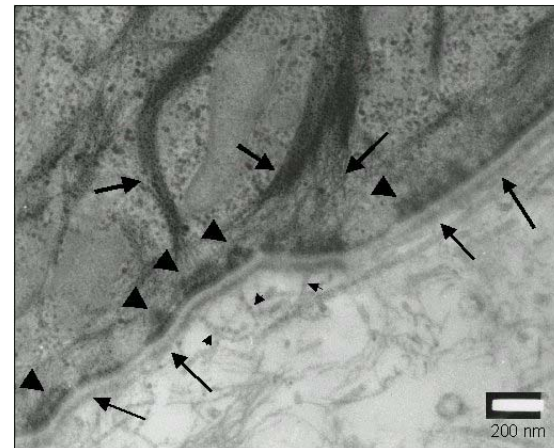
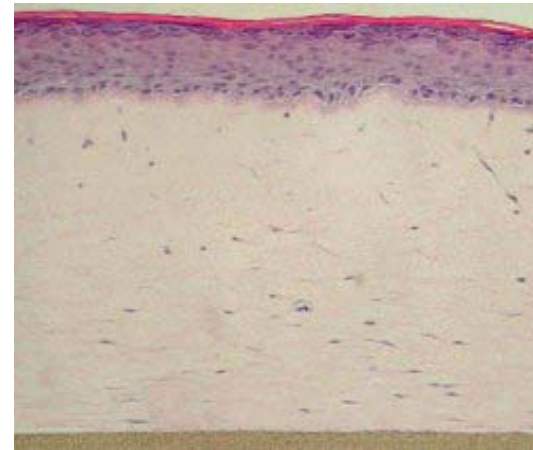
- Normal Human Keratinocytes
Cocultured with Normal
Human Melanocytes
 - Melanocytes from Caucasian,
Black or Asian Donors
- Exhibits Macroscopic
Darkening or Lightening in
Response to Chemical or
Biochemical Treatments
- Melanosome Transfer to
Keratinocytes

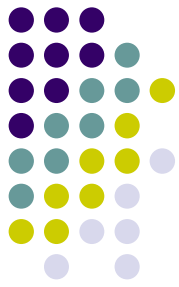


EpiDermFT™ Dermis/ Epidermis Human Skin Equivalent



- Dermis
 - Normal Human Fibroblasts in Collagen matrix
- Epidermis
 - Normal Human Keratinocytes Cultured on Top of Dermis
- Fully Developed Basement Membrane
- Defined Medium
- Applications
 - Wound Healing
 - Skin Aging/Photoaging
 - Keratinocyte/Fibroblast Interactions
 - Matrix remodeling



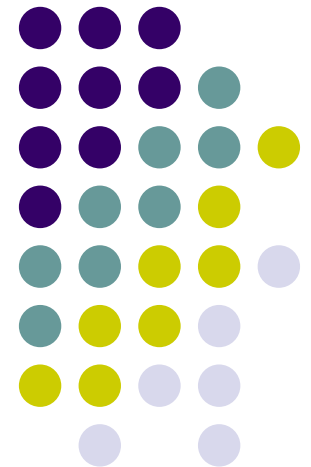


Summary of Section II

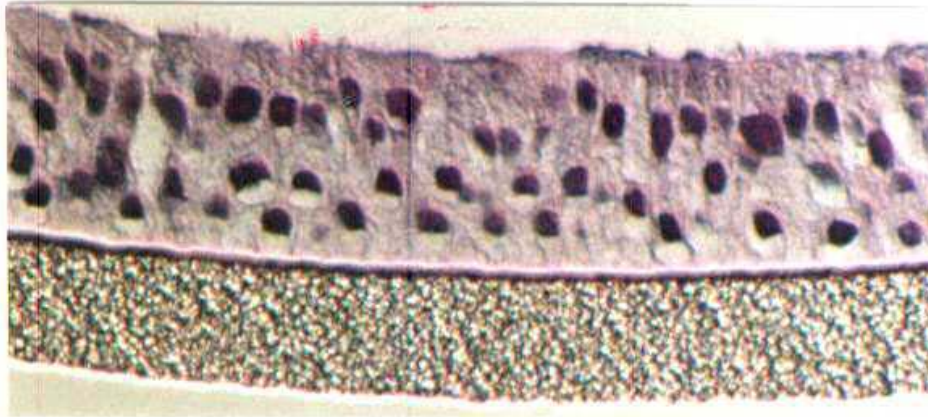
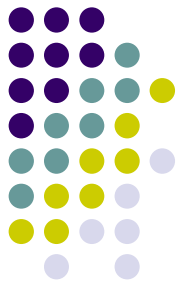
- Models of Epidermal, Epidermal/Dermal, Ocular, Tracheal/Bronchial, Ecto-cervical Vaginal, Buccal and Gingival have been produced.
- ALI Culture Allows Testing Under More Realistic Conditions.
- Normal (Non-Transformed) Cells, Not Cell Lines
- Human-Based Models Avoid Problematic Interspecies Interpolation.
- Differentiated Structure as well as Physical and Biochemical/Molecular Function

Section III

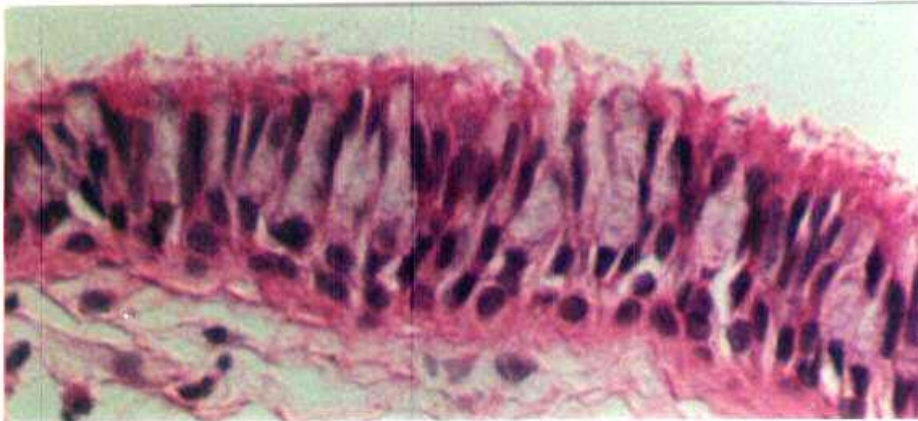
**ADME/TOX Applications of an
Organotypic Normal Human
Airway Epithelial Model for
Nasal Drug Delivery**



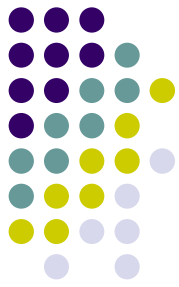
EpiAirway: In Vitro Human Tracheal/Bronchial Epithelium



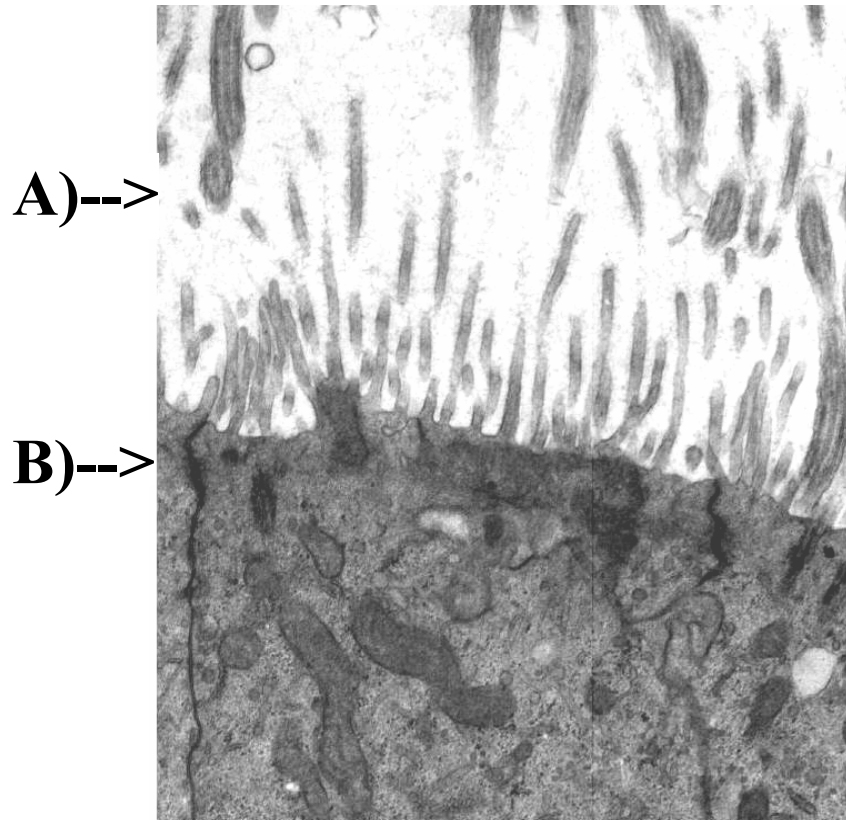
**EpiAirway
(AIR-100)**



**Human
bronchiole**



EpiAirway: Ultrastructure



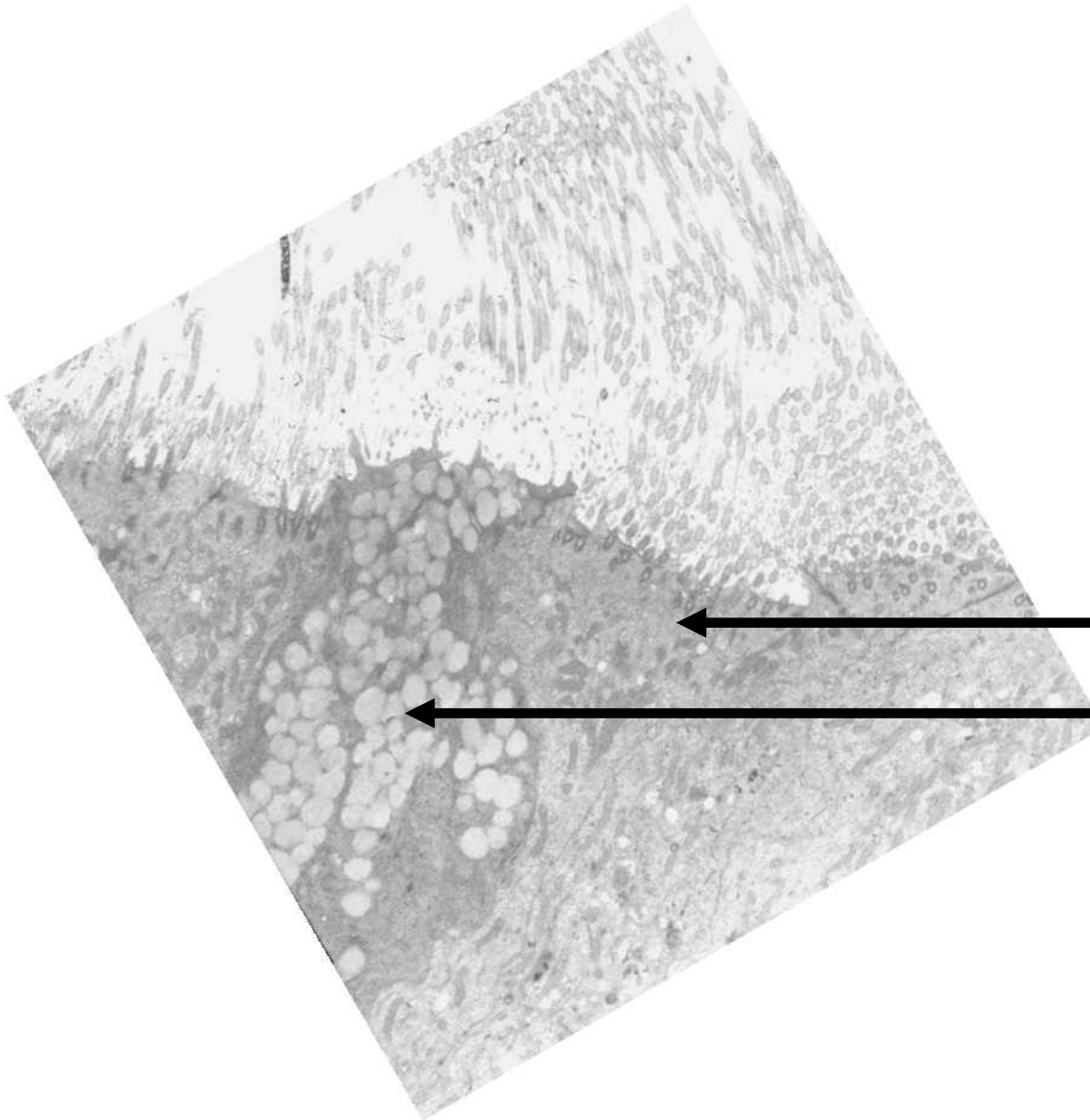
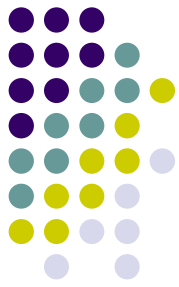
TEM micrograph:

A) Cilia on apical surface

B) Tight junctions

Magnification = 20,000X

EpiAirway: Ultrastructure

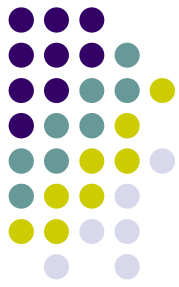


TEM micrograph:

Ciliated epithelial cells

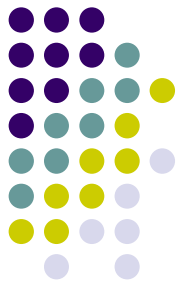
**Goblet cells –laden
with mucin**

EpiAirway™ Utilization in ADME/TOX Applications

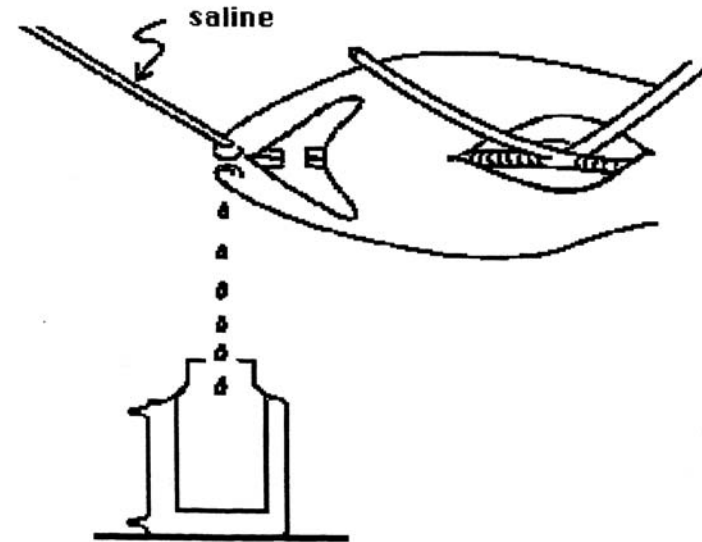


- Nasal Drug Delivery
 - Comparison to Rat In Vivo Model
 - Comparison to Excised Bovine Nasal Epithelium
 - Pre-Clinical Nasal Formulation Optimization
- Respiratory Infection Studies
- Inhalation Toxicology
- Role of Airway Epithelium in Airway Inflammation and Disease

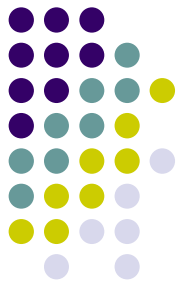
Traditional In Vivo Rat Model for Nasal Drug Permeation Studies (1)



- Very Good Correlation to Human Clinical Results in Most Cases
- Peptide Metabolism in Humans is Not Modeled Well in Rats
- Requires Surgical Procedures on Rats In Vivo

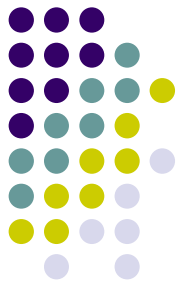


Comparison of EpiAirway to Rat In Vivo Model for Drug Permeation (2)

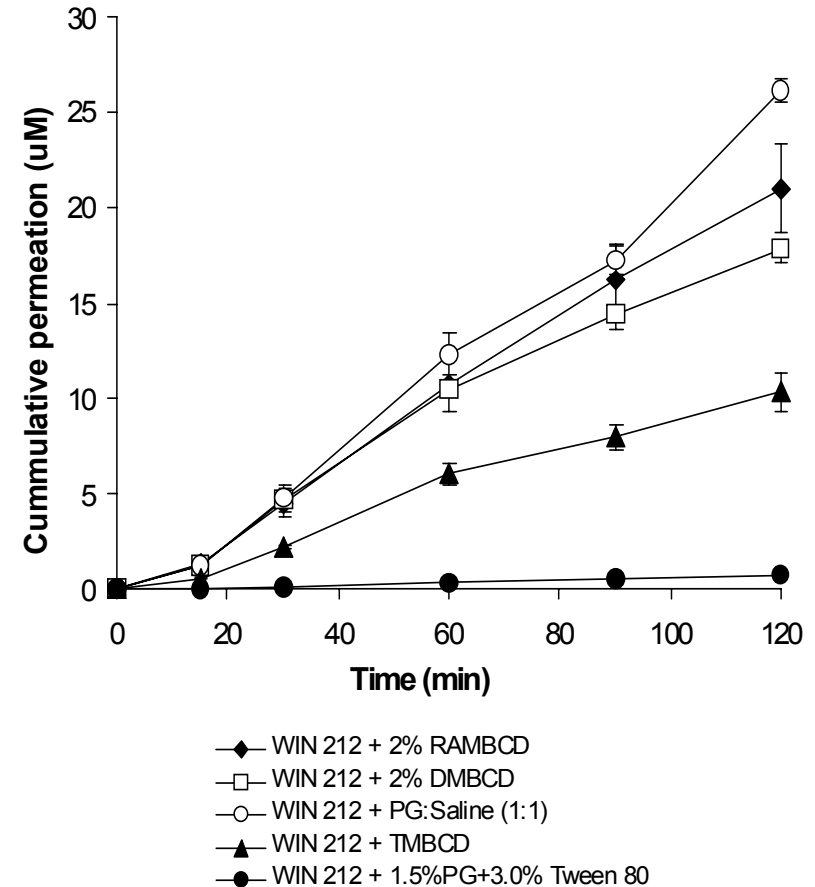


- Permeation of WIN 55,212-2, a cannabinoid receptor agonist
- Formulations containing propylene glycol and cyclodextrins
- Permeability coefficients in EpiAirway compared to rate and extent of absorption in the rat model in vivo.

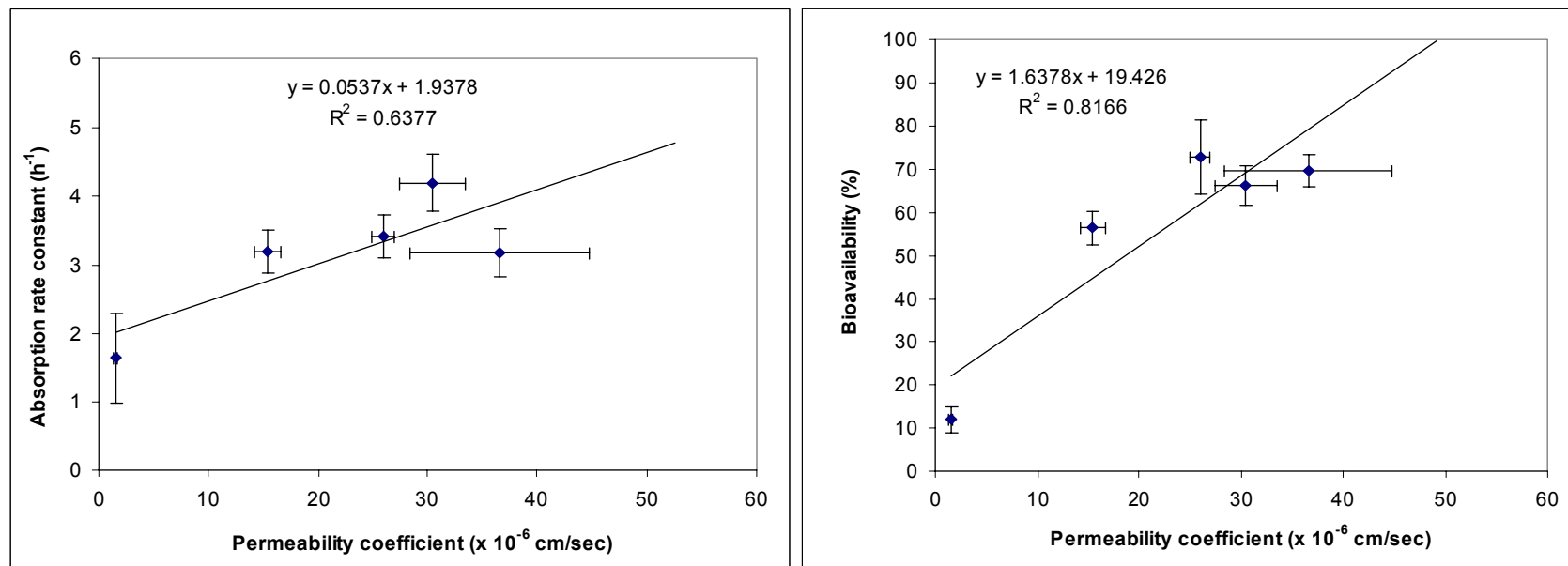
Permeation of WIN 55,212-2 in Various Formulations in EpiAirway (2)



- Permeation of WIN 55,212-2 across EpiAirway™ tissues.
 - RAMβCD, randomly methylated-β-cyclodextrin;
 - DMβCD, dimethyl-β-cyclodextrin;
 - PG, propylene glycol;
 - TMβCD, trimethyl-O-betacyclodextrin.
- Data represent mean ± SD, n = 3.

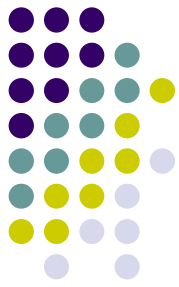


Correlation of EpiAirway In Vitro Model to Rat In Vivo Surgical Model (2)



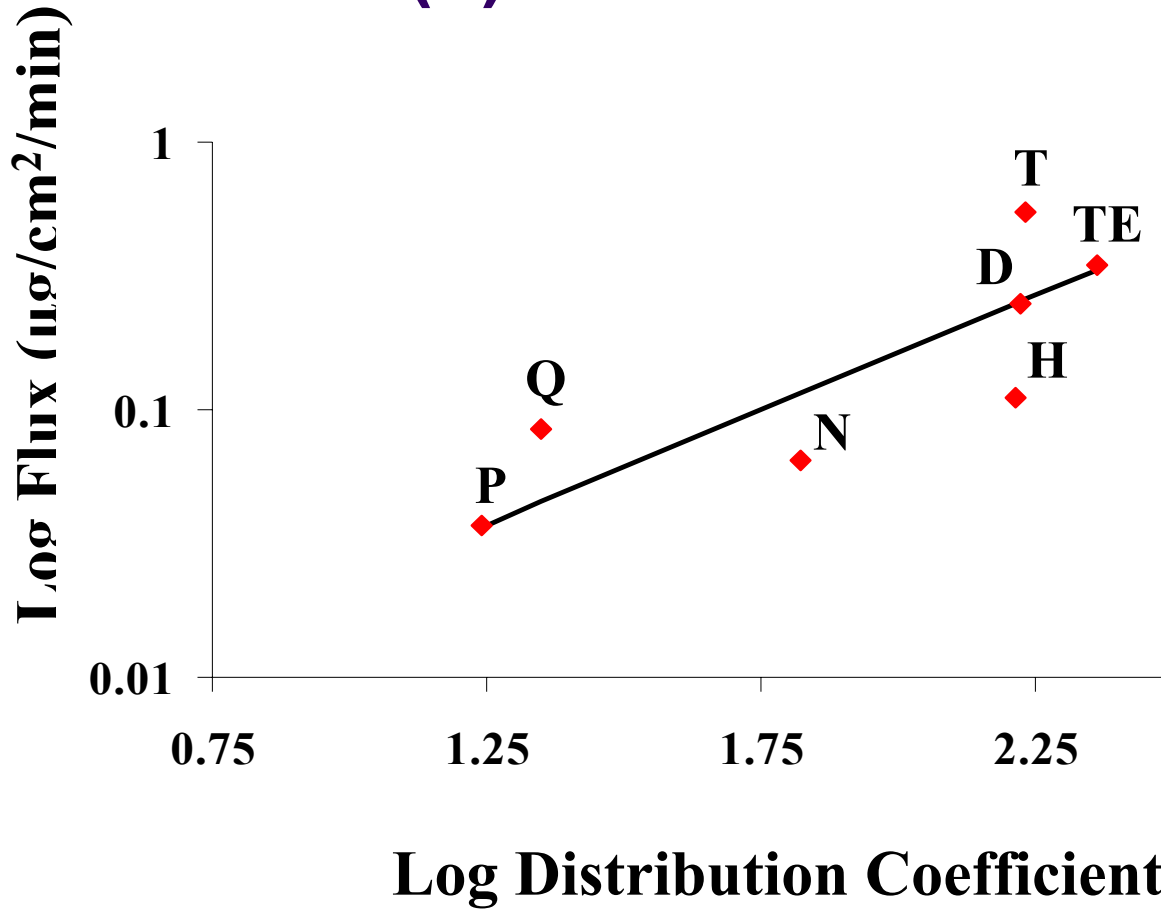
Relationship between permeability coefficients of WIN 55,212-2 across human EpiAirway™ tissues and its rate (A) and extent (B) of absorption in the rat nasal surgical model. Each point represents mean \pm SD ($n = 3$); R^2 , correlation coefficient.

Correlation of EpiAirway In Vitro Model to Excised Bovine Nasal Tissue (3)



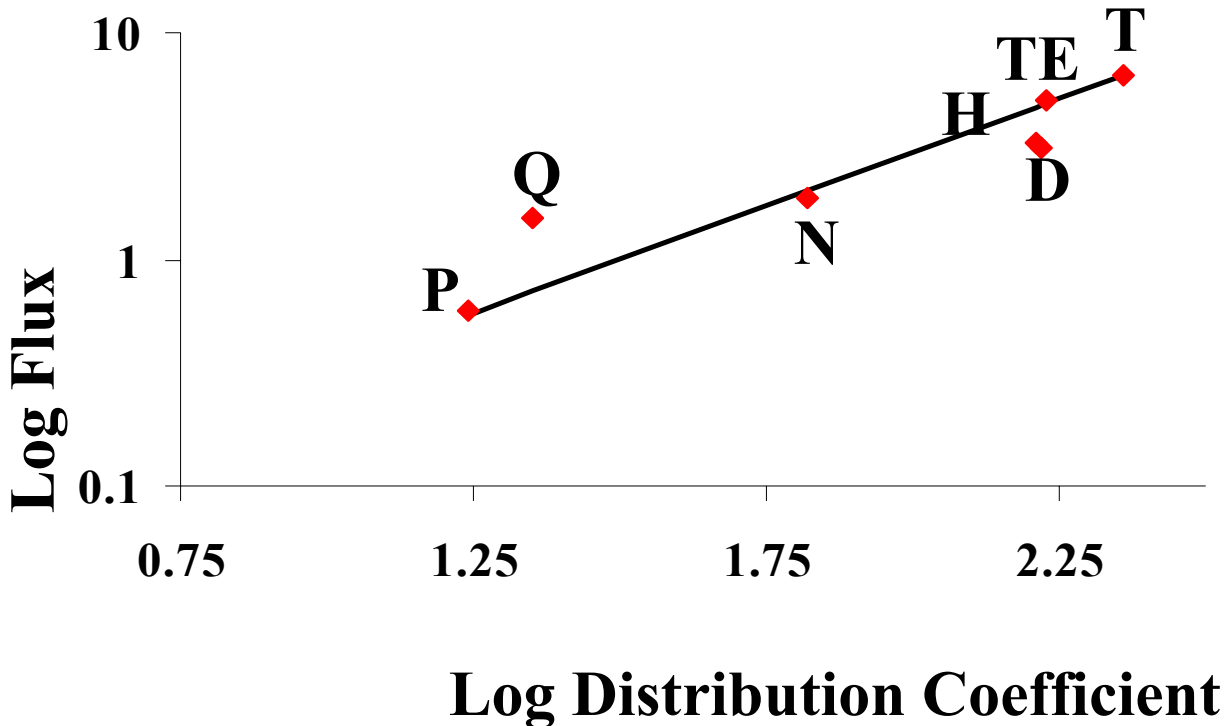
- Excised bovine nasal tissue is another popular model for nasal drug permeation studies
- Flux of compounds of various distribution coefficients tested in excised bovine tissue model compared to EpiAirway

Effect of Distribution Coefficient on Flux Across Bovine Respiratory Mucosa (3)



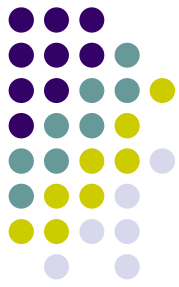
- P-** Phenol Red
- Q-** Quinidine HCl
- N-** Naloxone HCl
- H-** Hydroxyzine 2HCl
- D-** Dextromethorphan HBr
- TE-** Tetracaine HCl
- T-** Triprolidine HCl

Effect of Distribution Coefficient on Flux Across EpiAirway™ System (3)



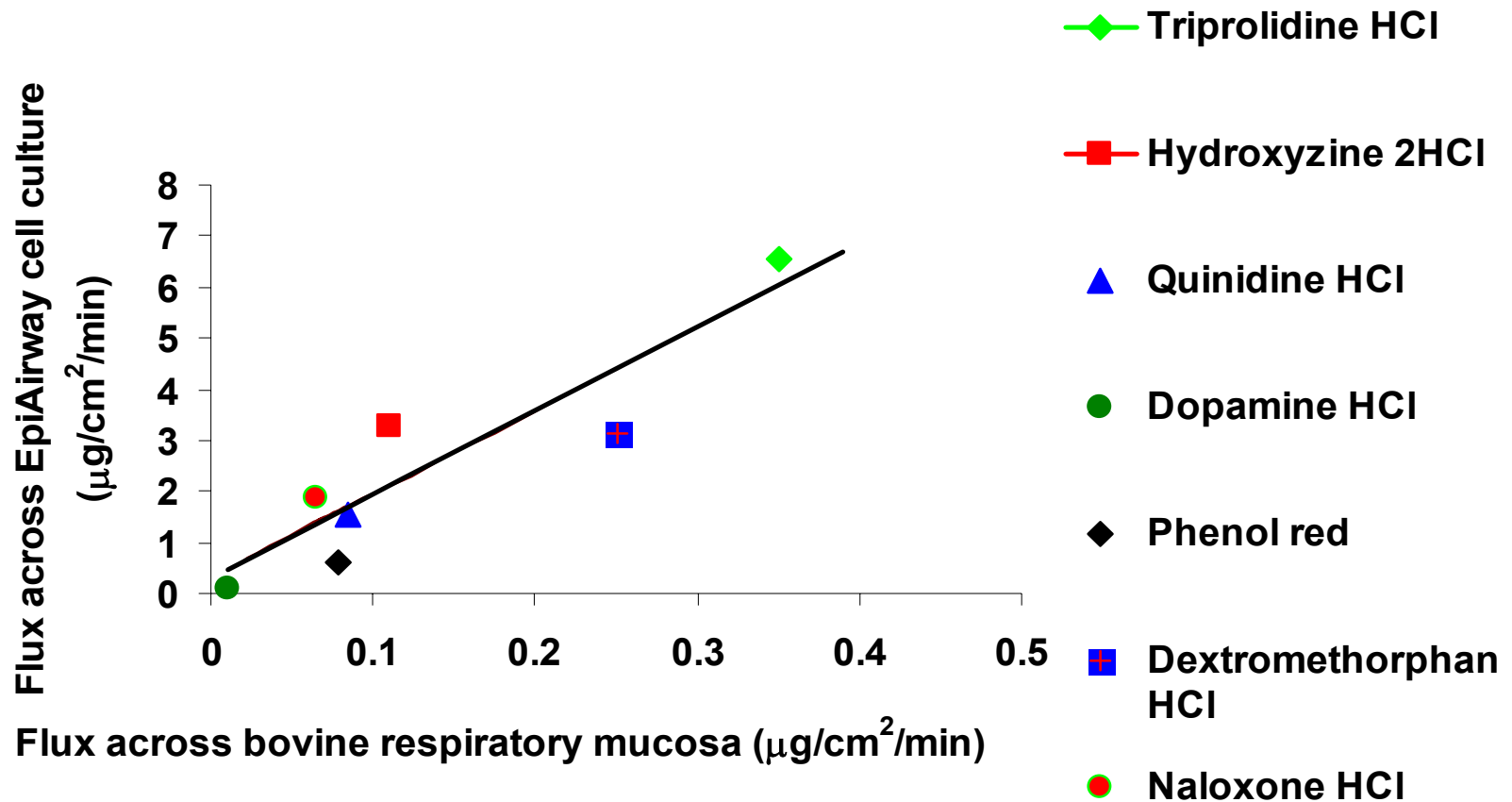
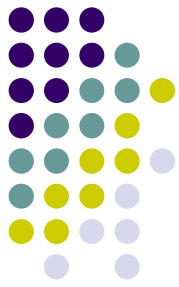
- P-** Phenol Red
- PR-** Propranolol HCl
- Q-** Quinidine HCl
- N-** Naloxone HCl
- H-** Hydroxyzine 2HCl
- D-** Dextromethorphan HBr
- TE-** Tetracaine HCl
- T-** Triprolidine HCl

Flux Across Bovine Nasal Explants and EpiAirway™ System (3)

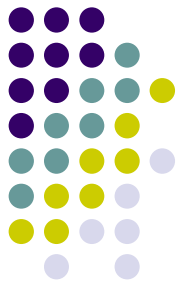


Compound	Flux ($\mu\text{g}/\text{cm}^2/\text{min}$)		Log D.C.
	Respiratory mucosa	EpiAirway™ system	
Triprolidine HCl	0.35	6.57	2.36
Tetracaine HCl	0.548	5.04	2.23
Dextromethorphan HCl	0.25	3.13	2.22
Hydroxyzine 2HCl	0.11	3.27	2.21
Naloxone HCl	0.065	1.85	1.82
Quinidine HCl	0.085	1.53	1.35
Phenol Red	0.037	0.59	1.24
Propranolol HCl		3.54	0.99
Dopamine HCl	0.01	0.1	-2.36
Cefazolin HCl	0.47	0.358	-2.87

Comparison of flux between bovine respiratory mucosa and EpiAirway™ system (3)

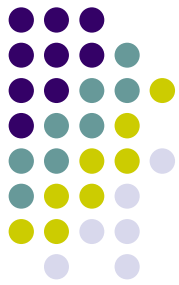


Pre-Clinical Nasal Formulation Optimization (4,5)



- **Interferons (MW = 20,000) Formulated with Permeation enhancers (3,4)**
- **Experimental Endpoints**
 - **Transepithelial electrical resistance (TEER)**
 - **Tissue Viability**
 - **Permeability measurements**

Pre-Clinical Nasal Formulation Optimization (4,5)



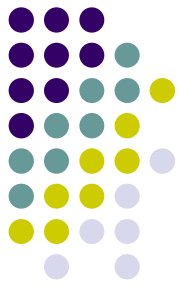
<i>Interferon alpha-2b</i>			
	TEER	Viability	Permeation
<u>Enhancer</u>	<u>% Decrease</u>	<u>(% of control)</u>	<u>Increase (fold)</u>
NF5	0	100	3
NF6	6	100	3
NF4	50	100	3
NF9	78	100	25
NF2	84	91	37
NF8	85	89	8
NF10	92	53	14
NF3	95	84	25
NF7	97	80	34
NF1	99	81	135

Pre-Clinical Nasal Formulation Optimization (4,5)



<i>Interferon beta-1a</i>			
	TEER	Viability	Permeation
<u>Formulation</u>	<u>% Decrease</u>	<u>(% of control)</u>	<u>Increase (fold)</u>
NF16	38	99	2
NF15	80	52	15
NF17	99	39	25
NF18	98	13	69
NF19	99.5	97	85
NF20	99.5	51	319

Summary of EpiAirway™ Utilization in ADME/TOX Applications



- Correlates well with rat in vivo and excised bovine nasal mucosa models for drug uptake studies (2,3)
- Useful for optimization of nasal drug formulations (4,5)
 - Endpoints measured
 - TEER
 - Viability
 - Drug penetration
- Bacterial adhesion studies (6)
- Inhalation toxicology studies (2,4,5,7)
- Airway inflammation and disease (8,9)



References

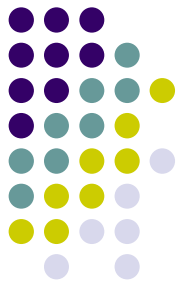
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