Using Mucosal Tissue to Evaluate Effectiveness

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Drug development pathway

**Stage 1**
- Drug discovery
- 10,000 compounds
- 6.5 years

**Stage 2**
- Pre-clinical
- First clinical trial application submitted
- 25 – 40 people

**Stage 3**
- Clinical trials
- Phase I (safety)
- Phase II (efficacy)
- Phase III
- 200–400 people
- 3000–10000 people
- 5 compounds
- 7 years

**Stage 4**
- Regulatory review
- Marketing application submitted
- 200–400 people
- 3000–10000 people
- 1 approved drug
- 1.5 years

Adapted from: Pharmaceutical Research and Manufacturers of America, 2006
What we’ll talk about...

- Pre-clinical microbicide testing
- Tissues used for explant cultures
- What can we do with tissue explants?
- Moving toward validation
Pre-clinical testing

- Traditionally done by testing the compound for anti-HIV activity and cellular toxicity
- Primary immune cells or cell lines for high throughput testing
- Handful of laboratories using mucosal tissue explant cultures
Why use mucosal tissues?

- Mucosal tissue is where the virus enters and where the product will be applied.
- Tissue consists of relevant cell types (HIV targets) in biologically appropriate ratios.
- Surgical remainders:
  - do not require patient recruitment.
  - obtained within a few hours of surgery.
- Endoscopic/colposcopic biopsy:
  - require patient recruitment.
  - obtained within minutes of scoping and convenient.
What tissues are used?

- Colorectal
  - Endocervix
  - Endometrium

- Ectocervix
  - Vagina
  - Penis
  - (glans, foreskin)
  - Anus
Colorectal and ectocervical models

- Colorectal tissue – obtained from surgical resections or endoscopic biopsies
  - Non-polarized (Anton/McGowan, Shattock)
  - Polarized (Dezzutti)
- Ectocervical tissue – obtained from pre-menopausal women undergoing hysterectomies
  - Non-polarized (Asin, Shattock)
  - Polarized (Dezzutti, Gupta [with T cell co-culture])
Non-polarized colorectal explants

- 2 mm piece of colorectal tissue
- Explants exposed to virus/microbicide while submerged in media (96 well plate)
- Explant placed on a media-soaked gelfoam raft in a 24-well plate following viral exposure
- Cultured in DMEM/pen/strep/±10% FCS
- Infection determined by presence of p24 in culture supernatants (10-14 days post exposure)

Fletcher, P.S. AIDS 20:1237, 2006
Non-polarized colorectal explants

Endoscopic biopsies + Absorbable gelatin sponge

Fletcher, P.S. AIDS 20:1237, 2006
Polarized colorectal explants

- 5 mm circular piece of colon (biopsy punch) and muscle is excised
- Explant is placed (epithelium on top) on presoaked gelfoam inserted into a transwell
- Explant is sealed with Matrigel around the epithelium

Abner, S. J. Infect. Dis. 192:1545, 2005
Polarized colorectal explants
Non-polarized ectocervical explants

- Human cervical tissue: cut into 2-3mm “explants” (complete RPMI)
- Submerged tissue exposed to virus (2h)
- Wash
- Culture overnight

- Transfer explants to fresh plates
- Culture for 12-14 days to determine infection of explants (p24 in culture supernatant)

Greenhead, P. J. Virol 74:5577, 2000
5 mm circular piece of ectocervix (biopsy punch) and muscle excised
Explant inserted through a hole in filter of a transwell insert and sealed with Matrigel around the epithelium

Polarized ectocervical explants
Non-polarized vs. polarized

Non-polarized

Pros
- Utilize all tissue
- Perform more replicates
- Models “worst case” scenario

Cons
- Can not evaluate transmission events
- Can not evaluate formulations

Polarized

Pros
- Biologically relevant
- Allows apical application of product/virus
- Model topical and systemic application

Cons
- Limits tissue utilization
- Limited replicates
What can we do with explants?

- Evaluate drug and/or formulation safety
  - MTT assay
  - Histology
  - Drug permeability
- Determine product efficacy
- Ex vivo product testing
Product safety – formulated

Microbicide

MTT assay (mitochondrial activity)

Histology
Product safety – formulated

MTT assay

% Viability of Control Tissue

TFV 1% Vehicle Control TFV 1% Vehicle Control N9

Ectocervical Colorectal

Product safety – formulated

Histology

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>TFV 1%</th>
<th>Vehicle Control</th>
<th>N9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectocervical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td></td>
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</tbody>
</table>

3 of 5 tissues exhibited facture or sloughing of the epithelium

What can we do with explants?

- Evaluate drug and/or formulation safety
  - MTT assay
  - Histology
  - Drug permeability
- Determine product efficacy
- Ex vivo product testing
Drug permeability

- Tissue is placed between donor and receptor chambers
- Product is added to donor chamber
- Receptor chamber is sampled at designated time points
TFV permeability

What can we do with explants?

- Evaluate drug and/or formulation safety
  - MTT assay
  - Histology
  - Drug permeability
- Determine product efficacy
- Ex vivo product testing
Product efficacy – formulated

Microbicidal or API

HIV p24 ELISA

Immunohistochemistry for p24 at study endpoint – ectocervix only

HIV
Product efficacy

Product efficacy

- In the presence of semen
- Model coital independent product use
## Testing with semen

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Osmolality (mmol/kg)</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% semen</td>
<td>8.14</td>
<td>321</td>
<td>3.96</td>
</tr>
<tr>
<td>50% semen*</td>
<td>8.11</td>
<td>311</td>
<td>2.19</td>
</tr>
<tr>
<td>20% semen*</td>
<td>8.10</td>
<td>310</td>
<td>1.56</td>
</tr>
<tr>
<td>1% TFV gel</td>
<td>4.45</td>
<td>3347</td>
<td>3979.3</td>
</tr>
</tbody>
</table>

* diluted in DMEM
Testing with semen

Dezzutti Lab, unpublished data
Testing with semen

Histology

Control

100% semen

50% semen

N9

Colorectal

Ectocervix

Dezzutti Lab, unpublished data
Testing with semen

Permeability of $^3$H-$\text{H}_2\text{O}$ in Excised Human Tissue

- $^3$H+Semen
- Control $^3$H+DMEM

Rohan Lab, unpublished data
Testing with semen

- No detectable semen enhancing viral infectivity (SEVI) effect

![Graph showing HIV-1 p24gag levels in culture over days of culture with and without semen](image)

Dezzutti Lab, unpublished data
Testing with semen

Dezzutti Lab, unpublished data
Gel is applied for 1 h, 24 h before (pre) or after (post) exposure to HIV-1.

2 of 10 explants not protected regardless of semen

4 of 18 explants not protected regardless of semen

Dezzutti Lab, unpublished data
What can we do with explants?

- Evaluate drug and/or formulation safety
  - MTT assay
  - Histology
  - Drug permeability
- Determine product efficacy
- Ex vivo product testing
Ex vivo testing

Anton, P., McGowan, I. Poster CROI 2009

V2: Baseline; V3: 30 minutes post single dose
Ex vivo testing

- Non-polarized vs. polarized explants

IHC positive: 88% non-polarized
71% polarized

Lynam, JD, Poster M2010
Explants can be used to...

- Evaluate microbicide safety and efficacy
  - Drug permeability
  - In the presence of semen
  - Multiple formulation types (gel, film, ring)

- Determine ex vivo efficacy
  - Surrogate for clinical efficacy?
Caveats of using explants

- Expensive and cumbersome to obtain
- Independent of hormonal control
- Inability to regenerate/repair
- No vascularization
  - No recruitment of immune cells

Therefore, explants should be used
  - for the most promising candidates (not for screening)
  - as part of a comprehensive testing algorithm
MTN pre-clinical algorithm

Formulated Product

Formulation Testing
- Osmolality, pH, viscosity, in vitro release

In vitro Testing
- Dose Range
  - Cell lines
  - Lactobacillus
- HIV efficacy
  - ± human secretion

Ex vivo Testing
- Cervical/colorectal tissue
- Absorption, permeability, and safety
- HIV efficacy
  - ± human secretion

Human Studies
Preclinical testing picture
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