# Generation and Characterization of an *in vitro* Organotypic Foreskin Model to Study the Penile Microbiome

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CIHR IRSC Canadian Institutes of Health Research Instituts de recherche en santé du Canada

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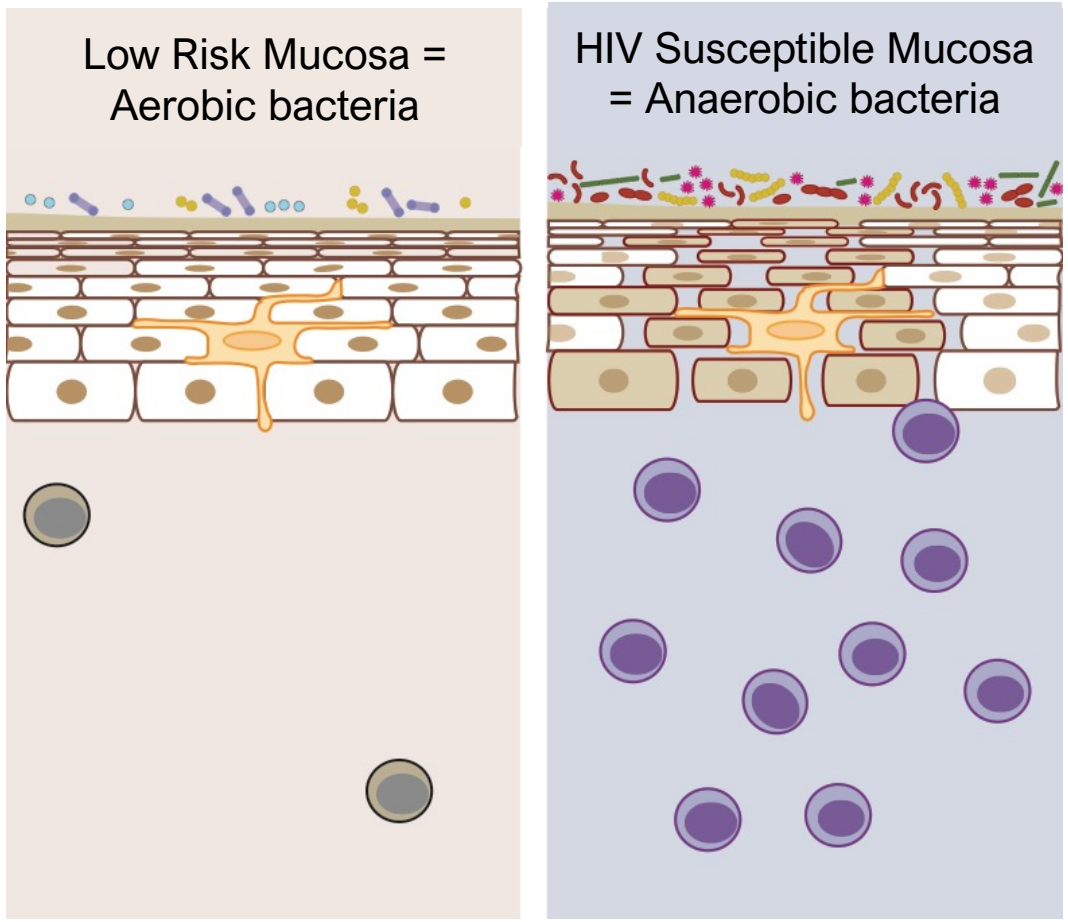
- Removal of the foreskin (circumcision) reduces HIV susceptibility by up to 60% in heterosexual men
- Circumcision causes profound shifts in the foreskin microbiome, such as decreasing in penile anaerobe abundance
- Foreskin anaerobes are correlated with immune cell density, inflammatory cytokine production, and HIV susceptibility




## Problem

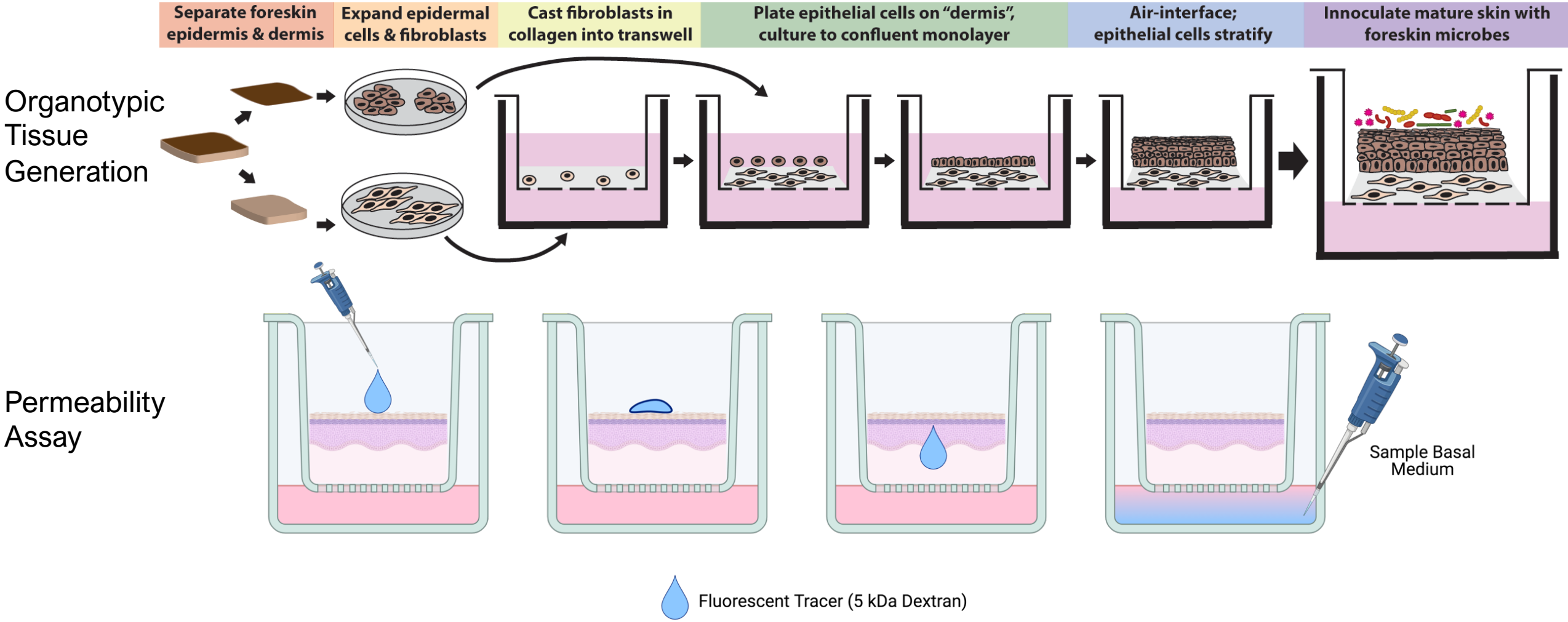
- Studies examining HIV and anaerobes are all observational
- Foreskin microbes co-occur, so cannot discern mechanism of action for HIV susceptibility or which anaerobes are high-risk
- No method to empirically show causal relation between anaerobes and HIV susceptibility

## Solution

- Generate 3D *in vitro* organotypic foreskin tissue model
  - Mimics pertinent skin aspects
  - Allows one to tease apart which microbes are involved in HIV susceptibility through bacterial co-culture methods
  - Can establish causal relationships, develop targeted antimicrobials

Liu *et al.*, 2017

- *In vitro* organotypic foreskin models will be generated using primary cells from patients undergoing circumcision
- Tissue models will be examined for markers of differentiation and ability to exclude fluorescent dextran compared to commonly used skin cultures (explant cultures)



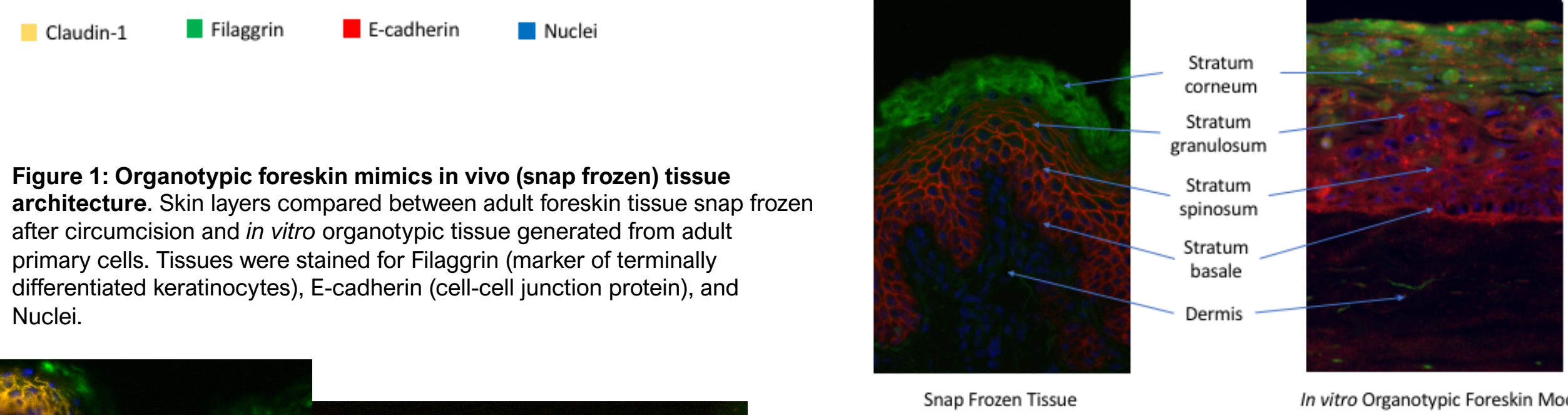
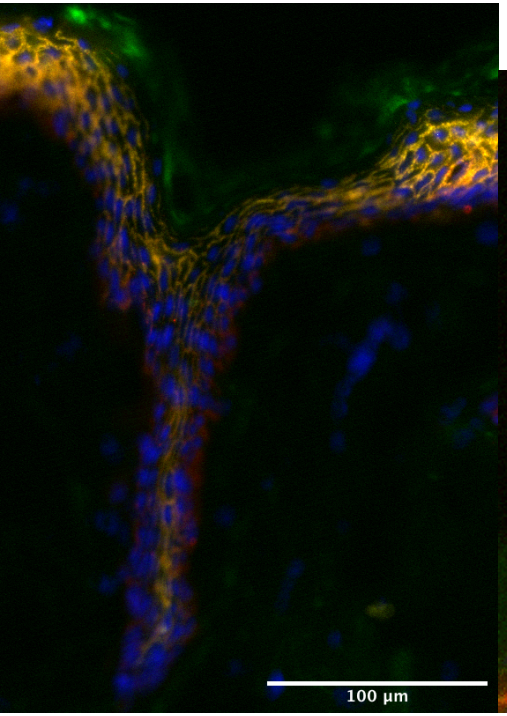
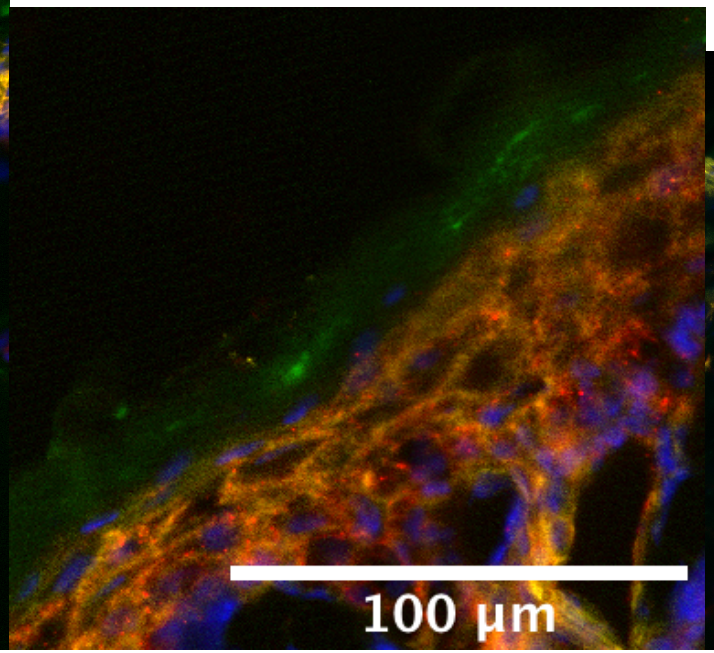


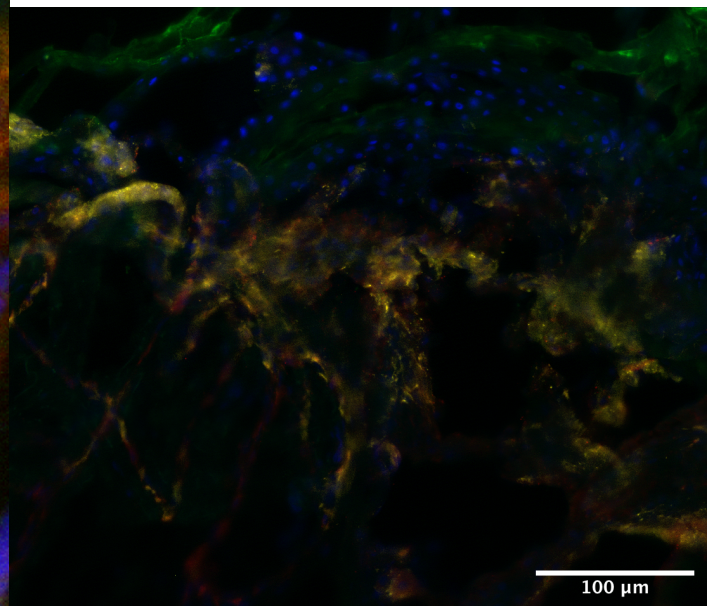

**Figure 1: Organotypic foreskin mimics in vivo (snap frozen) tissue architecture**. Skin layers compared between adult foreskin tissue snap frozen after circumcision and *in vitro* organotypic tissue generated from adult primary cells. Tissues were stained for Filaggrin (marker of terminally differentiated keratinocytes), E-cadherin (cell-cell junction protein), and Nuclei.




4-hour Cultured Foreskin




24-hour Cultured Foreskin




7-Day Cultured Foreskin

**Figure 2: Cultured explant tissue loses tissue architecture after a one-week culture period**. Representative images of pediatric foreskin tissue subject to tissue culture for different periods of time (n=3 tissues). Tissues were stained for Claudin-1 (tight junction protein), Filaggrin, E-cadherin, and Nuclei.

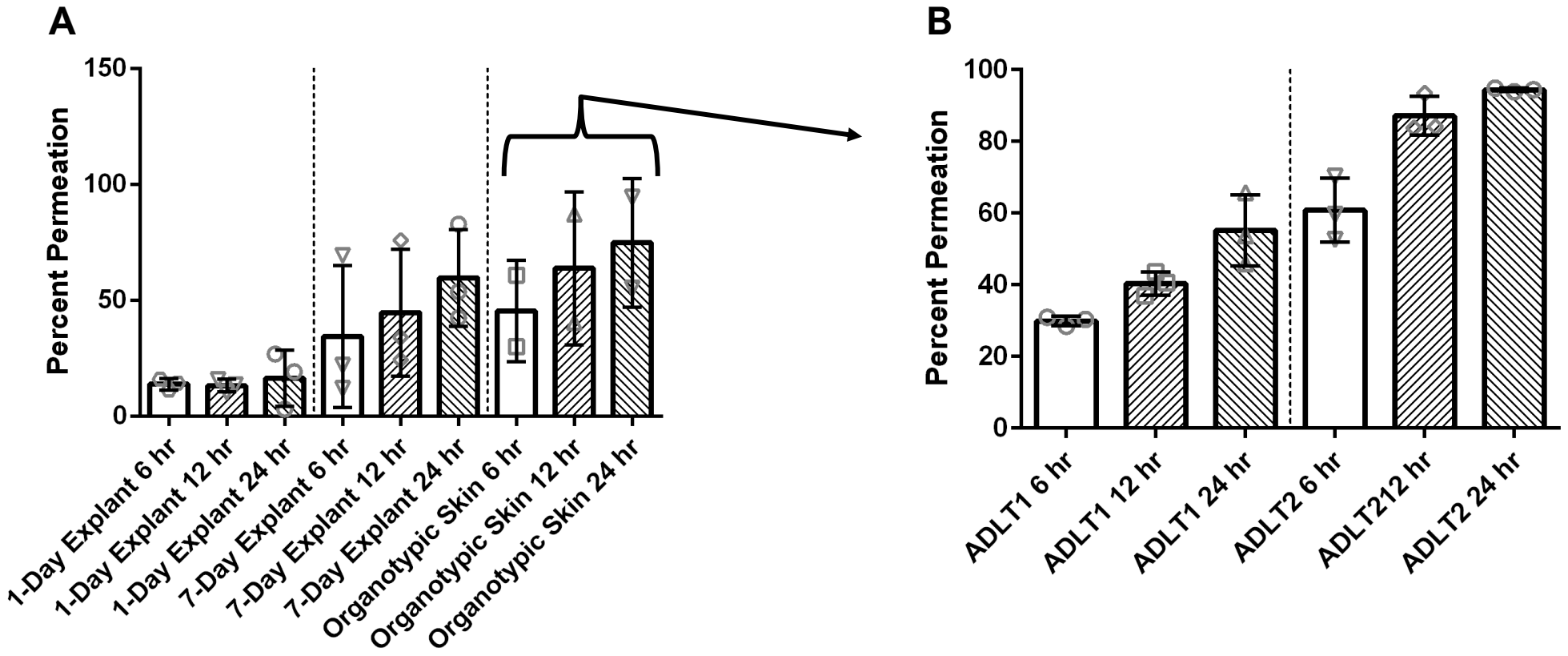



**Figure 3: Tissue permeability across explanted and organotypic foreskin tissues**. 5 kDa fluorescent dextran was added to the apical surface of skin tissues, with readouts of tissue culture medium fluorescence at 6, 12, and 24 hours. **A**: Percent permeation of paediatric skin cultured for 1-day and 7-days (n=3 tissues) alongside organotypic foreskins (n=2 tissues) grown using adult foreskin cells. **B**: Percent permeation between organotypic tissues from two different adult patients (n=3 technical replicates) showing high variability between two generated samples.

- While fluorescent staining suggests the organotypic model mimics *in vivo* skin, and that this tissue can exist in culture for more than one week, more work is still required to ascertain organotypic tissue permeability.