

## Organotypic Human Small Intestinal Tissue to Assess Epithelial Restitution

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Presented at AAPS NERDG Annual Meeting, May 1, 2014, Farmington, CT

### Abstract

**Purpose:** The intestinal epithelium (SMI) **forms** an important physiological barrier in the gastrointestinal tract but this barrier can be compromised by a wide range of substances including drugs, microbes, and dietary substances traveling through the lumen. Since repeated epithelial damage or injury are implicated in intestinal disorders including inflammatory bowel diseases, rapid closure or resealing of wounds has key physiological importance. In this study, we describe an *in vitro* SMI model cultured using normal human intestinal cells which closely recapitulates the physiology and function of the small intestine to study epithelial restitution.

**Methods:** Normal human primary SMI epithelial cells, fibroblasts, and endothelial cells were expanded in monolayer culture and seeded onto transparent microporous membrane inserts to reconstruct the 3D SMI tissues. Injury was induced on the 3D tissues using 1 or 2 mm biopsy punches. The injured tissues were analyzed daily for epithelial restitution using phase contrast microscopy, confocal imaging of migrating epithelial cells (immunostained for cytokeratin 19) and fibroblasts (immuno-stained for vimentin), transepithelial electrical resistance (TEER) measurements to monitor recovery of epithelial barrier integrity, and H&E staining to examine the level of wound closure and re-epithelialization.

**Results:** Following injury, TEER of the SMI tissues dropped from 160  $\Omega \cdot \text{cm}^2$  (baseline) to 55  $\Omega \cdot \text{cm}^2$ . On day 1 after the injury, fibroblasts became more visible in the wound area and epithelial cells shouldering the wound began to migrate into the wounded area. Confocal and H&E imaging of injured tissues showed cooperation of fibroblasts and epithelial cells in the wound healing process. Wounded areas not resealed by epithelial cells were initially covered by fibroblasts. Overall, completion of wound healing was achieved in 4-6 days post-injury. On days 4-6: 1) the migrating epithelial cells resealed the wound and migrating epithelial cells re-polarized (confirmed by confocal imaging and H&E staining) and 2) tissue barrier returned to pre-injury, baseline levels (TEER). **Conclusions:** The newly developed SMI tissue model will likely be useful for testing candidate drugs or biologics to treat diseases that are characterized by injuries of small intestine epithelial barrier.

## Methods & Results

**Tissue preparation:** Small intestine (SMI) epithelial cells harvested from post-mortem donors following IRB approval. SMI cells were seeded onto cell culture inserts (partial thickness tissue, SMI-100) or onto a myofibroblast collagen-gel matrix (full-thickness tissue, SMI-100-FT), raised to the air liquid interface and cultured in specially formulated culture medium designed to induce differentiation for 2 weeks. A representative crosssection of the organotypic SMI tissue model is shown in **Figure 1**.

**Histology:** To examine structural features small intestinal epithelial tissues were fixed in 10% formalin (overnight, room temperature), paraffin embedded, sectioned using a microtome, and stained with hematoxylin and eosin (H & E) according to standard procedures. The histological results revealed: 1) wall-to-wall growth of the epithelial layer (**Fig 1**), and 2) the presence of columnar epithelial cells similar to the in vivo counterpart.

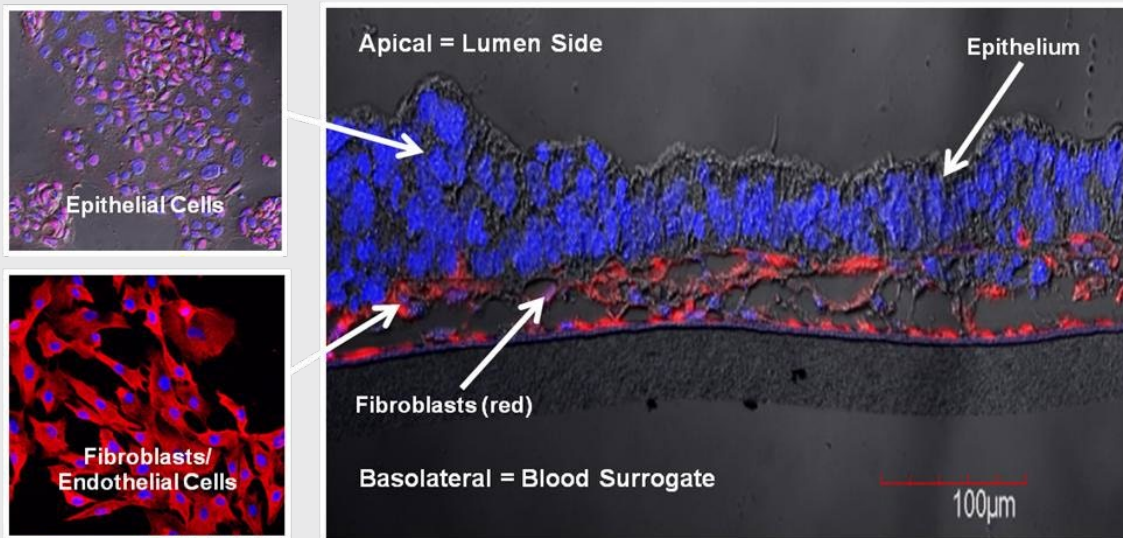
**Immunohistochemistry (IHC):** Immuno-staining was performed on formalin fixed SMI-100 tissues following antigen retrieval. Confocal imaging showed expression of ZO-1 and Claudin-1(**Figs 2 & 3**).

**Ultra structural features:** Transmission electron microscopy (TEM) was used to examine ultrastructural features such as brush borders and tight junctions in the small intestinal epithelial tissues (**Fig 4**).

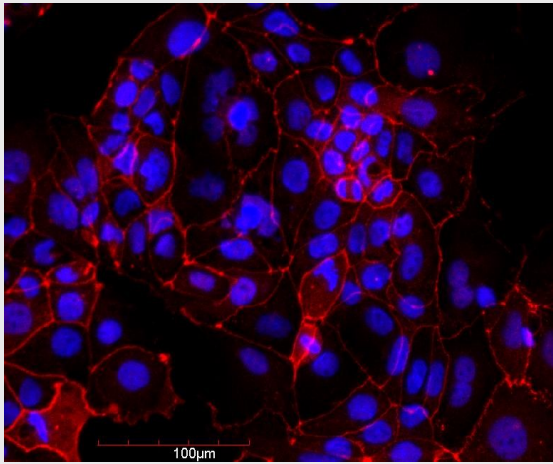
**Intestinal restitution:** Injury was induced in the 3D SMI-100-FT tissues using a 2 mm biopsy punch (severe injury; wound 25% of the tissue diameter; **Fig 5 and 6**) or 1 mm biopsy punch (mild injury; wound 10% of the tissue diameter; **Fig 7**). The injured tissues were analyzed daily for epithelial restitution using phase contrast microscopy, confocal imaging of migrating epithelial cells stained with cytokeratin 19 (**Figs 5-7**) and fibroblasts stained for vimentin (**Figs 6 & 7**),

**TEER and histology as markers of wound healing:** TEER measurements were used to monitor the recovery of epithelial barrier integrity after wounding of tissues (**Fig 8**). TEER measurements were made using an EVOM voltohmmeter equipped with an Endohm electrode chamber (World Precision Instruments, Sarasota, FL). TEER values (reported in  $\text{Ohm}\cdot\text{cm}^2$ ) were calculated by multiplying raw resistance measurements by the surface area of the tissue ( $0.6\text{ cm}^2$ ). H&E staining was also performed and showed re-polarization of epithelial cells and resealing of the wound at different days post-injury (**Fig 9**).

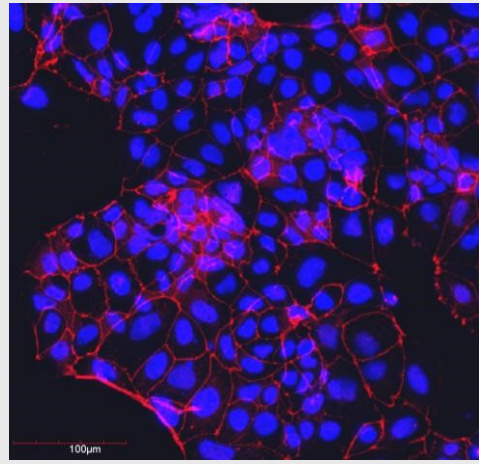
**Applications:** A schematic representation of the various applications of the intestinal tissue model is presented in **Fig.10**



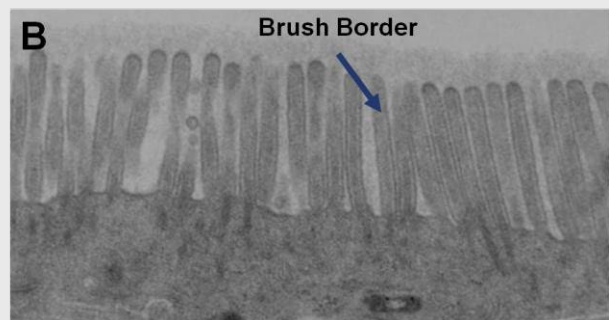
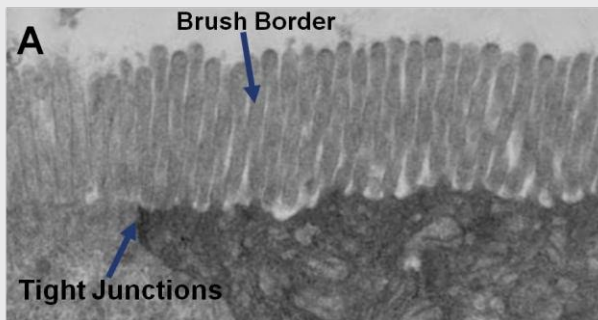
**Fig 1: Reconstruction of full-thickness EpilIntestinal tissue model (SMI-100-FT).** Endothelial cells, seeded on the underside of the membrane, are not shown in the above figure.



**Figure 2:** Confocal microscopy showing ZO -1 staining of the partial thickness (SMI -100) tissues.



**Figure 3:** Confocal microscopy showing claudin-1 staining of the partial thickness (SMI -100) tissues.



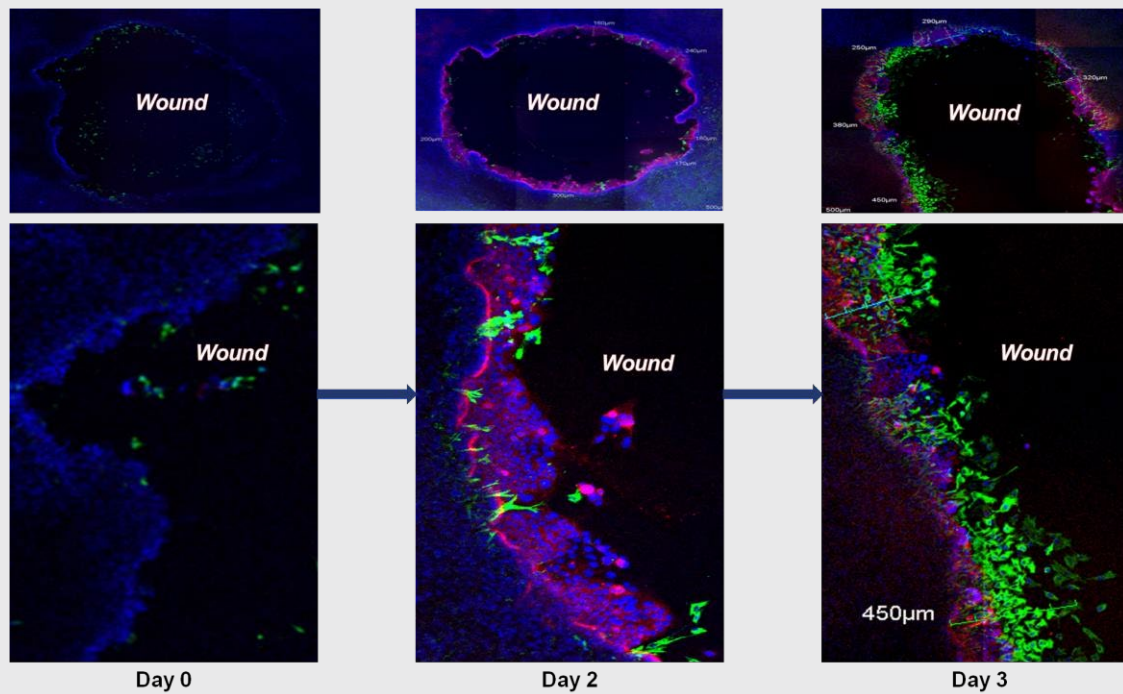
**Figure 4:** Transmission electron micrograph (TEM) of *in vitro* EpiIntestinal (A) and Explant tissues (B) showing Brush borders (situated at the luminal pole of the enterocyte) and tight junctions. *Brush border – provides digestive and absorption surface; site for enzymes & transporters.*



**Figure 5:** Immunohistochemistry showing restitution of organotypic 3D EpilIntestinal (SMI -100-FT) tissue after wounding with a needle tip (0.5 mm in diameter). Epithelial cells shouldering the wound migrate to reseal the injured tissue. Migrating epithelial cells express cytokeratin-19 (Red, see arrow); nuclei are stained with DAPI (Blue). *Note: No treatment was applied to enhance the restitution process.*

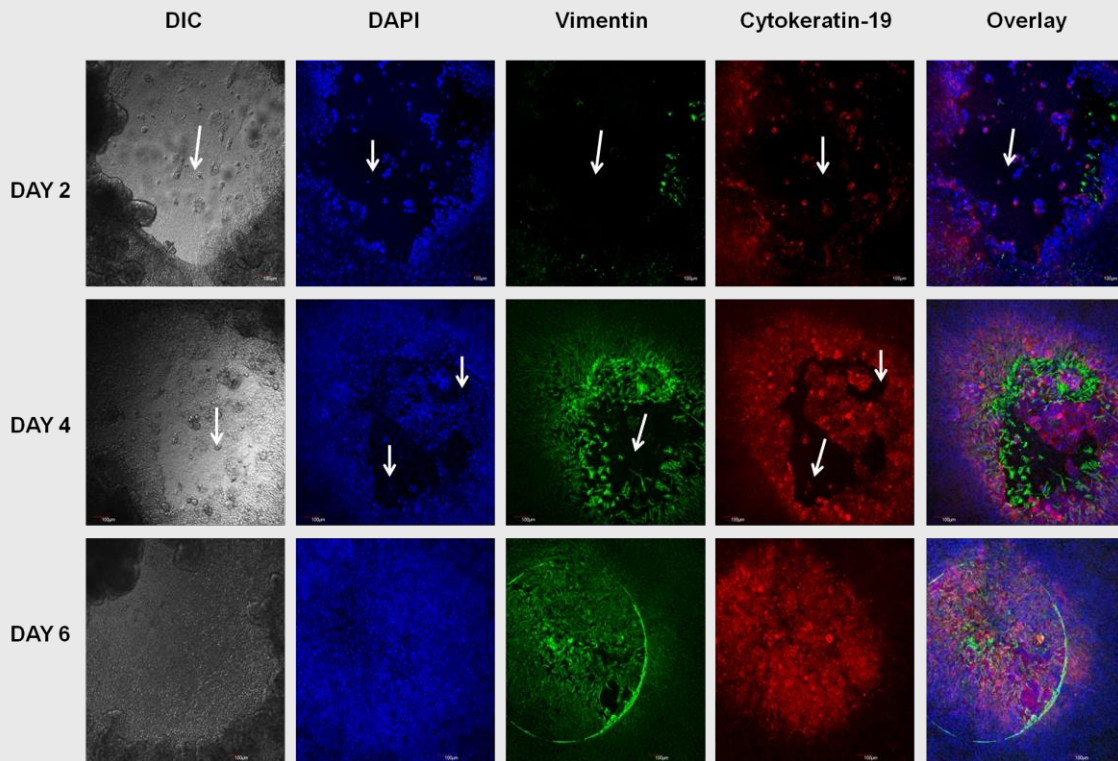


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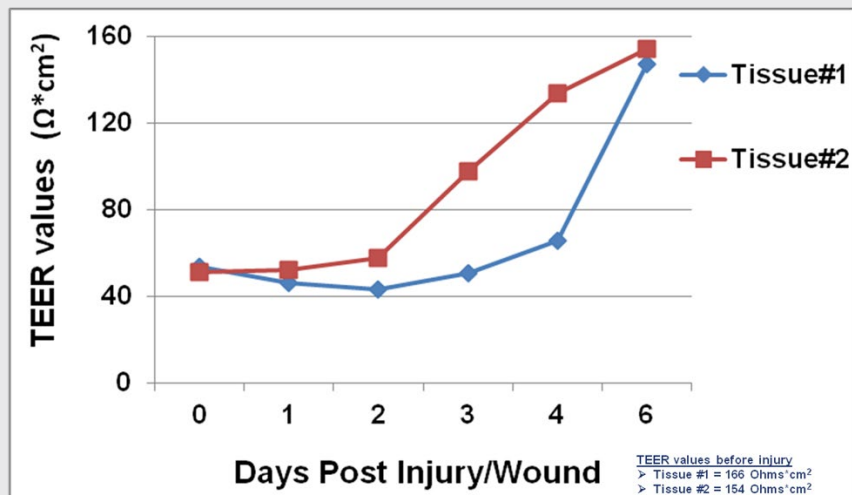


**Figure 6:** Restitution of SMI-100-FT tissue model 3 days after wounding with a 2 mm biopsy punch. Migrating epithelial cells are stained for cytokeratin-19 (red), fibroblasts for vimentin (green), and nuclei are stained with DAPI (blue). On day 3, the fibroblasts are at the leading edge of resealing the wound.

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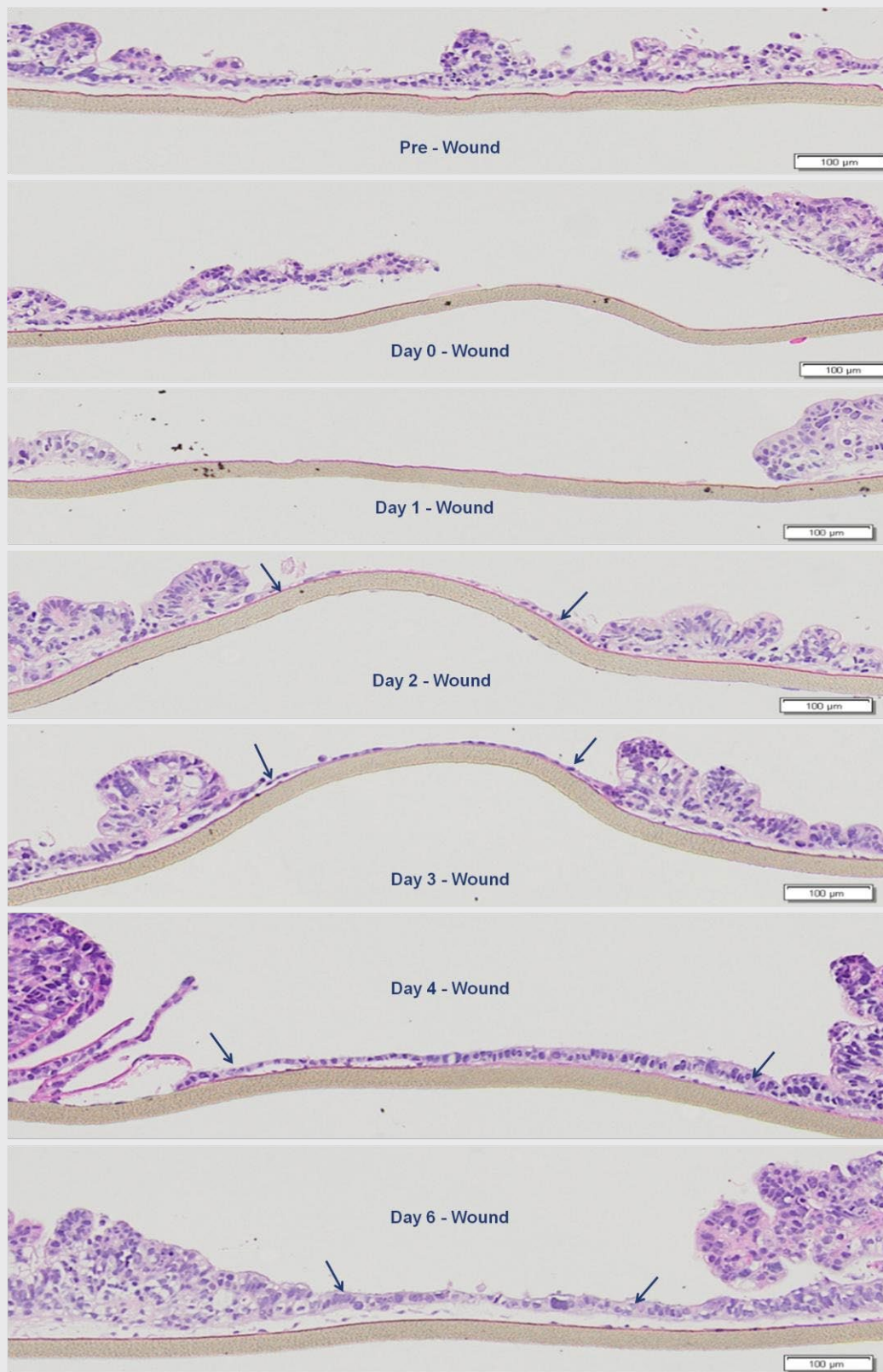


**Figure 7: Restitution of SMI-100-FT tissue model 6 days after wounding with a 1 mm biopsy punch.** Migrating epithelial cells express cytokeratin -19 (red); fibroblasts express vimentin (green), and nuclei are stained with DAPI (blue). On day 6, complete resealing of the injured tissue was complete.



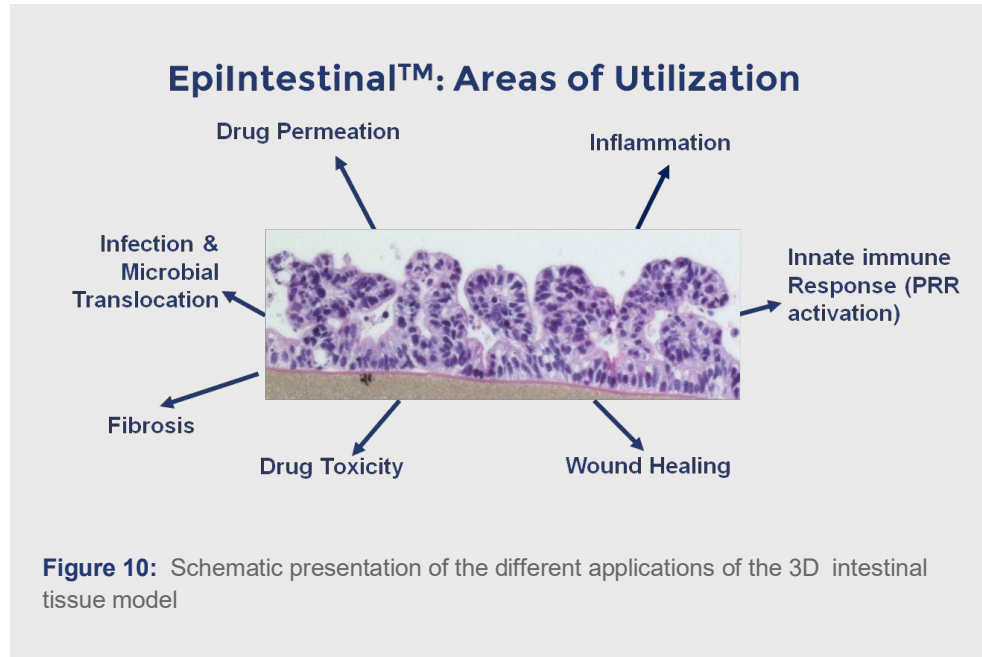
**Figure 8: TEER measurement showing recovery of epithelial barrier integrity following injury.**

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**Figure 9:** H&E staining showing epithelial restitution by the Epilntestinal tissue model. Arrow indicates migrating epithelial cells shouldering the wound.





## Summary and Conclusions

- Highly differentiated models of normal human small intestinal tissue have been developed. Microscopic and histological cross-sections show tissue structure that mimics in vivo intestinal tissue (**Fig 1**).
- Results showed that the reconstructed tissue model express tight junction proteins (**Figs 2 & 3**) and form brush borders and tight junctions (**Fig 4**).
- Confocal imaging showed cooperation of fibroblasts and epithelial cells in the wound healing process (**Figs 57**). The progress of wound healing could be following with barrier measurements (**Fig 8**) and histologically (**Fig 9**).
- The newly developed SMI tissue model will likely be useful for testing candidate drugs or biologics to treat diseases that are characterized by injuries of epithelial barrier.
- The SMI tissue model will also have application in drug safety, inflammation, fibrosis, and IBD studies and will also reduce animal use (**Fig 10**).