Washington University in St. Louis
SCHOOL OF MEDICINE

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Kyle Jewhurst, Ph.D.
Science Editor, JoVE

Dear Dr. Jewhurst,

Thank you for the invitiation to author a protocol for consideration at JoVE. We are please to submit our original research article entitled "**Modeling Intestinal Development and Injury-Repair In Vitro using Mouse Colonic Stem Cell Monolayer Culture under Air-Liquid Interface**" to be considered for publication at JoVE.

Intestinal epithelial stem cells are an important model of stem cell biology and cellular renewal during injury and repair. Existing 3D organoid systems are powerful tools to study intestinal development but are limited in the study of injury-repair because they provide only a snapshot of a specific cellular state. In this protocol we present an approach to generate long-lived, self-organizing 2D monolayer cultures of mouse colonic epithelium that contains all epithelial cell types. We believe that this method will be of broad interest to your readers and accelerate future mechanistic studies of intestinal development, repair, and interactions with microbes and other cell types.

Based on our previously published and widely reproduced methods, we first demonstrate how to collect and expand mouse colonic stem cells. Next, we present a new detailed protocol to collect colonic stem cells and seed them on Transwell membranes as epithelial monolayers. When the monolayer is exposed to an Air-liquid interface, the cells undergo a rapid proliferative burst followed by cytodifferentiation of all colonic cell types including goblet, enteroendocrine, and absorptive cells. The monolayer self-organizes to resemble flattened colonic crypts with foci of proliferative stem cells interspersed amongst differentiated, non-proliferating cells. Importantly, this self-renewing monolayer is culturable for at least four weeks, which enables studies of long-term development as well as host-microbe and epithelial-mesenchymal interactions. Another key advantage of this system is the ability to model injury-repair cycles by resubmerging the monolayer after ALI. This leads to numerous cellular and functional changes so that the monolayer resembles an atrophic crypt, which is the pathological feature of intestinal damage. After re-establishing ALI, the monolayer returns to a homeostatic state, modeling the cycle of colonic injury and repair. We believe that this protocol will be an important tool for mechanistic studies of cellular renewal and translational models of human intestinal disease.

Sincerely,

Brian Muegge, M.D., Ph.D.

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