A Tri-Cell Culture Model of Human Intestine Constructed on Electrospun Polyurethane Scaffold Inserts in the MIVO® Millifluidic Device: Effect of Static vs. Dynamic Flow Culture Conditions

Maria E. F. Palamà[1], Maurizio Aiello[1], Silvia Scaglione[1], Patrick J. Hayden[2], Matthew D. Phaneuf[2]
[1]React4life S.p.A. Genoa 16152, Italy, [2]BioSurfaces, LLC, Ashland, MA 01721 USA

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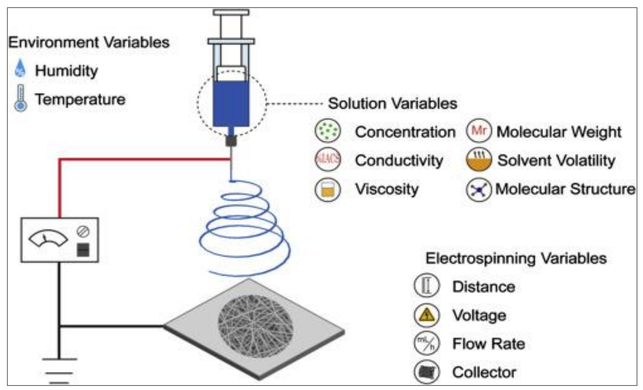


Figure 1. Basic process variables for creating electrospun scaffolds by solution electrospinning. Polymer solutions are dispensed across a high voltage field and collected on a grounded surface. Source: Kurecic, Manja. (2013). Electrospinning: Nanofibre Production Method. Tekstilec. 56. 4-12. 10.14502/Tekstilec2013.56.4-12.

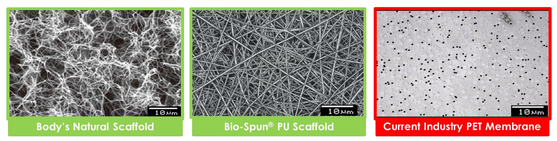


Figure 2. Scanning electron micrograph of *in vivo* extracellular matrix, Bio-Spun® PU scaffold, and a film-based microporous membrane. 3D randomly oriented nanofiber scaffolds are similar to 3D *in vivo* extracellular matrix. The film-based PET membrane is a highly rigid 2D surface.

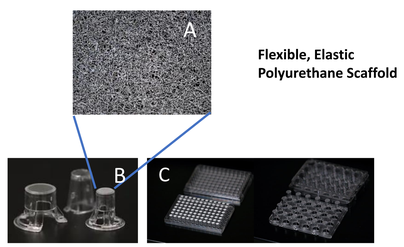


Figure 3. Electrospun scaffold insert products. Bio-Spun® scaffolds (A) are bonded to various sizes of individual inserts (B) and 24-and 96-well HTS plate format components (C). Inserts and plates are shown in the upside-down orientation to highlight the scaffold component. The HTS formats are compatible with robotic plate handlers and individual inserts are compatible with several common organ-on-a-chip fluidic systems.

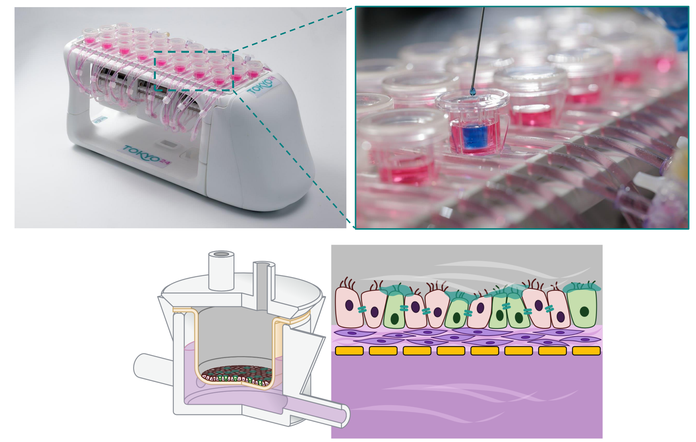
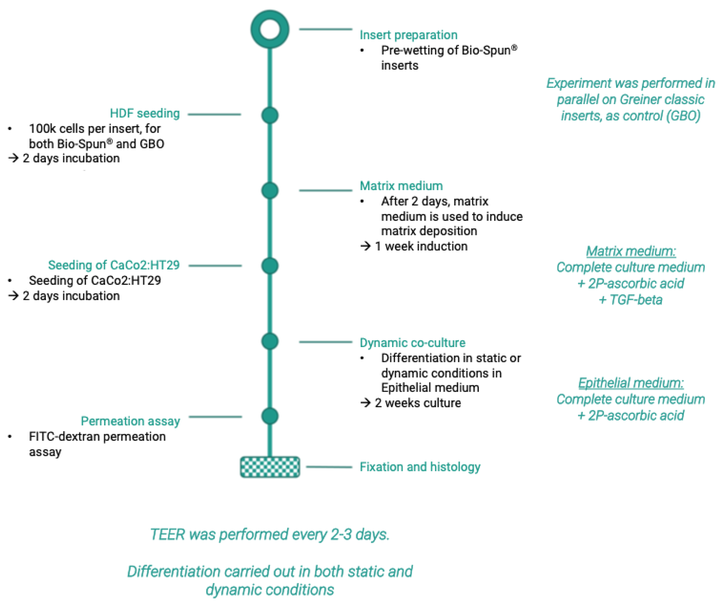


Figure 4. The MIVO® Millifluidic System. (A) MIVO® flow chamber assembled within the Millfluidic flow apparatus. (B) Tri-culture epithelial model contained within the MIVO® flow chamber

Background

Accurate *in vitro* modelling of the human gut is a key need for drug development and disease treatment. However, traditional *in vitro* models struggle to accurately replicate the gut's complex mechanisms. The aim of the current work is to develop an *in vitro* model using electrospun polyurethane scaffold inserts (Bio-Spun® PU, IIC24 301) on the MIVO millifluidic organ on chip device that more accurately reproduces the morphological and functional aspects of the human intestinal epithelium, including incorporation of dynamic *in vivo*-like basolateral perfusion to mimic interstitial fluid flow and peristaltic mechanical forces.

Experimental Outline



Results

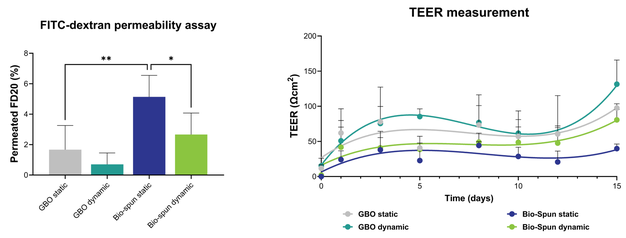


Figure 5. Barrier Assessment. (A) FITC-dextran permeability. Permeation <4% is considered to indicate healthy and differentiated intestinal tissue. (B) TEER. FITC-dextran permeability and TEER of dynamic cultures were statistically improved compared to static conditions.

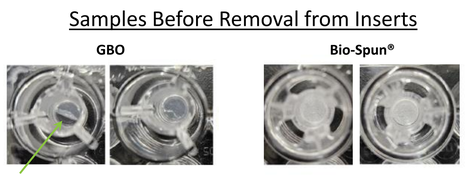


Figure 6. Tissue attachment to inserts during experiments and during handling. (G) Tissues cultured on Greiner inserts tended to contract and detach during the experiment and histological processing. (B) In contrast, tissues cultured on Bio-Spun® inserts did not experience contraction or detachment issues.

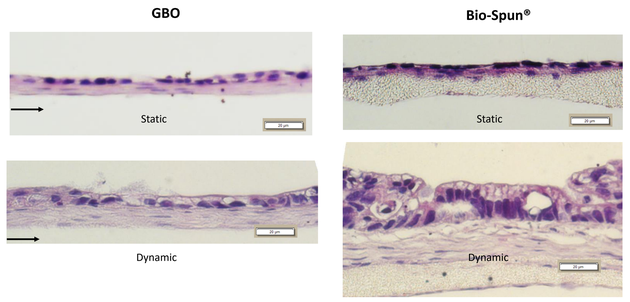


Figure 7. Histological morphology of intestine models cultured on Greiner and Bio-Spun® inserts under static *vs.* dynamic conditions. Note that tissue has detached from the Greiner membrane inserts (black arrows).

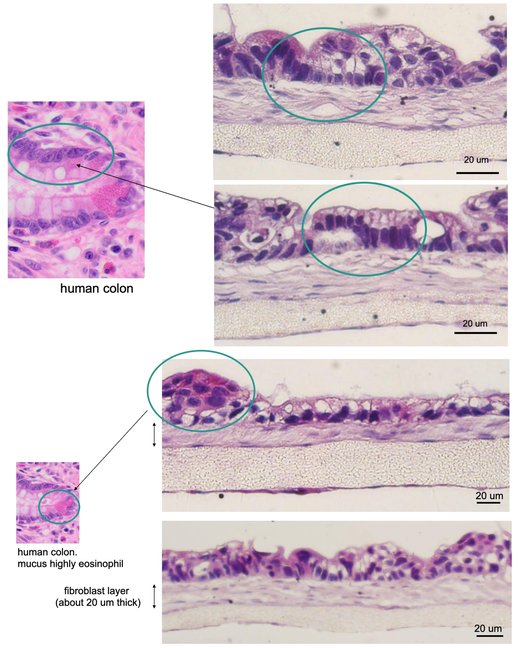


Figure 8. Dynamic culture conditions on Bio-Spun® scaffolds produced improved histological similarity with *in vivo* human mucosal colon epithelium.

Conclusions

1. Dynamic culture on flexible Bio-Spun® PU inserts improved tissue adhesion as well as barrier properties of HDF-CaCo-HT29 co-cultures as measured with TEER
2. Based on FITC-dextran permeation, HDF-CaCo-HT29 can be successfully differentiated on Bio-Spun® PU inserts, especially in dynamic conditions (permeation below 4%)
3. Intestinal tissue produced on the flexible Bio-Spun® PU inserts under dynamic culture showed improved differentiation with similar structural properties to human colon