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## Optimization of the permeation unit for advanced cell culture: Hostile territory for Caco-2 cells?

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Oral drug delivery remains the most preferred and convenient route of administration yet predicting in vivo drug absorption continues to present significant challenges. While conventional in vitro dissolution tests effectively assess drug release from formulations, they fail to fully capture the complexity of the gastrointestinal environment—such as intestinal metabolism, drug transport mechanisms, and epithelial barrier function—that critically influence absorption. Incorporating cell-based models, such as Caco-2 cells, into the permeation unit enhances the physiological relevance of in vitro studies.

To better simulate the in vivo absorption process, we optimized a custom-built dissolution-permeation apparatus for cell-based experiments. The original permeation unit, not intended for cell culture, required several key modifications. These included the implementation of gas exchange via 3D-printed polycarbonate vents with PTFE filters to prevent contamination, revised sterilization procedures to avoid material deformation, and the replacement of cytotoxic components with biocompatible alternatives. A tissue culture-compatible polycarbonate membrane was introduced and coated with collagen to promote cell adhesion.

Two cultivation strategies were investigated: static and dynamic. In the static setup, initial failure to form a cell monolayer was attributed to toxicity from brass plugs and sealing grease, which were subsequently replaced with PVC-U plug and PTFE tape, enabling successful Caco-2 cell culture. The dynamic system holds promise but requires further optimization, particularly in identifying tubing materials durable enough to withstand long-term flow conditions.