

Review of: "Characterization of an engineered mucus microenvironment for in vitro modeling of host–microbe interactions"

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This work proposes the construction of a system composed of a hydrogel layer (alginate/mucin) and an immiscible aqueous two-phase system of dextran/polyethylene glycol polymers (ATPS) on a monolayer of cells (16-HBE or Caco-2 cells) to model *in vitro* host-microbe interactions by providing a realistic mucosal microenvironment. However, the following questions arise:

- It is proposed here to form a system of three to four aqueous phases composed of different polymers and placed on the surface of two different cell types. However, the structure of this system and the interactions between its components has not been characterized.
- The rheological properties of the hydrogel formed by the mixture of alginate and mucin in the ATPS have not been evaluated. The diffusion properties of the antibiotics tested in the hydrogel (alginate/mucin) layer with the ATPS were also not evaluated. The macroscopic properties of the material, such as pore size and viscosity may be different in the proposed three/four-phase aqueous system relative to the hydrogel composed by the mixture of alginate/mucin. Therefore, the conclusions obtained here are questionable.
- The authors suggest that the presence of lipids in the crude mucin mixture creates a more hydrophobic surface for ATPS. However, in this work, the commercial mucin type III was used, which does not have lipids in its composition.
- Cell viability studies with Caco-2 cells are absent.