Development of a medium-to-high throughput organoid and bacteria co-culture platform for the assessment of host pathogen interaction

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Introduction

HUB´s patient derived organoids, (HUB Organoids® or PDOs) are self-organizing epithelial cell structures with near-physiological features, extensively used to model aspects of cancer initiation and progression. Microinjection of colibactin-producing pks+ *E. coli* into the lumen of PDOs results in the appearance of two co-occurring mutational signatures identified in a subset of colorectal cancer (CRC) patients[1], demonstrating that pks+ *E. coli* plays a causative role in CRC development. However, the scalability of bacteria microinjection in PDOs is limited and represents a bottleneck in the screening of preventive therapies for patients. Here we developed a bacteria and PDO co-culture system (PDO fragment exposure model), alternative to PDO microinjection, that is compatible with medium-to-high throughput screening methods. We validated the genotoxicity of colibactin-producing bacteria and showed the potential of the PDO fragment exposure model for the screening of drugs targeting colibactin-dependent genotoxicity.

Fig 1. Development of the first PDO

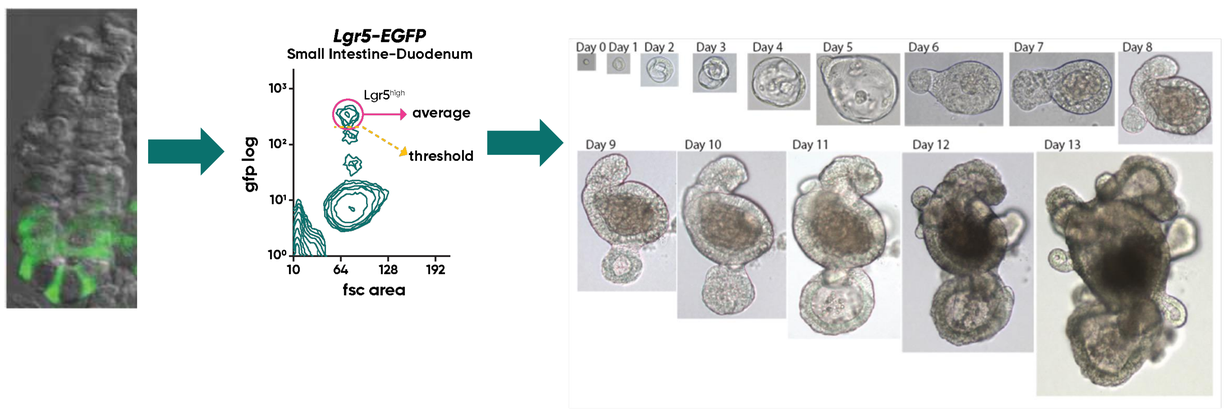


Fig 2. HUB Organoids for the development of therapies targeting pathogen-host interactions

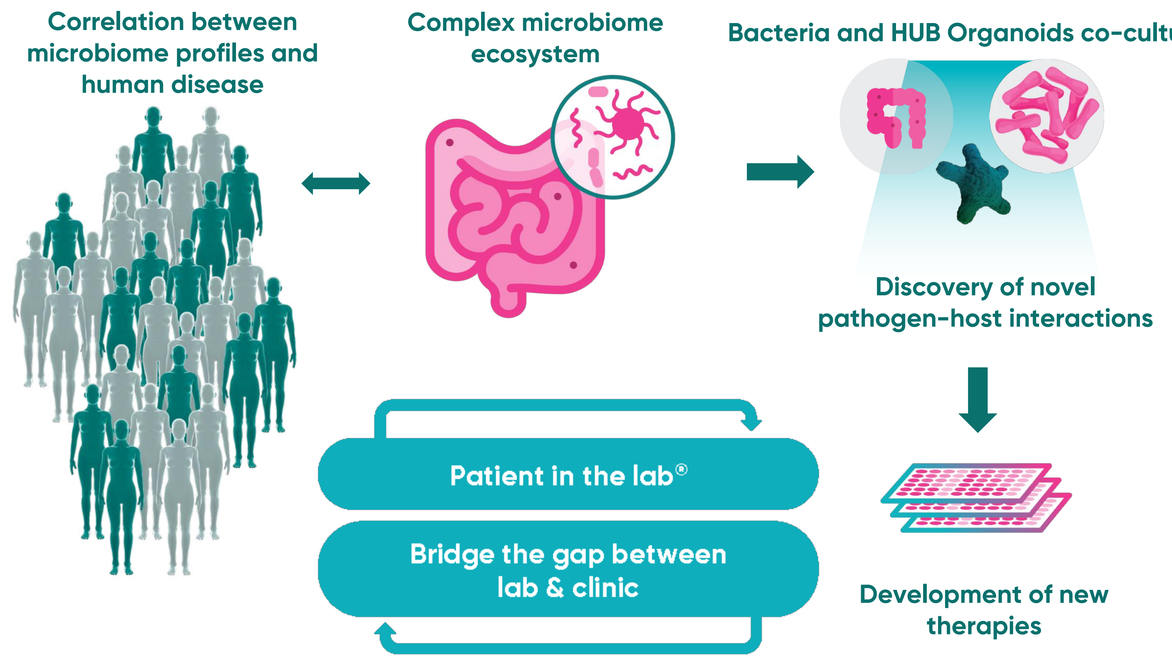
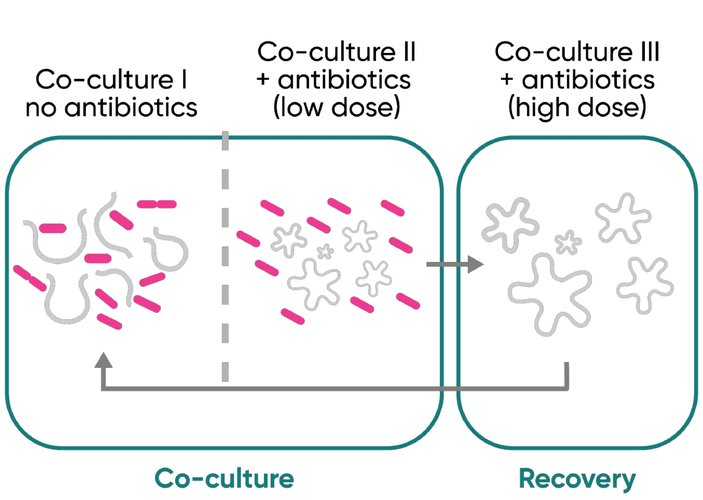


Fig 3. Strategy for PDO fragments and colibactin-producing bacteria co-culture

The PDO fragment exposure model consists of three phases: (a) Co-culture I or 'acute' DNA damage phase, where the bacteria and PDOs interact in suspension and the growth of the bacteria is not restricted; (b) Co-culture II or 'sustained' DNA damage phase, where PDOs and bacteria are cultured in hydrogels and bacteria growth is controlled by the addition of low concentrations of antibiotics; (c) Recovery phase, where the bacteria is killed by higher dose of antibiotics and PDOs continue to grow in hydrogels.



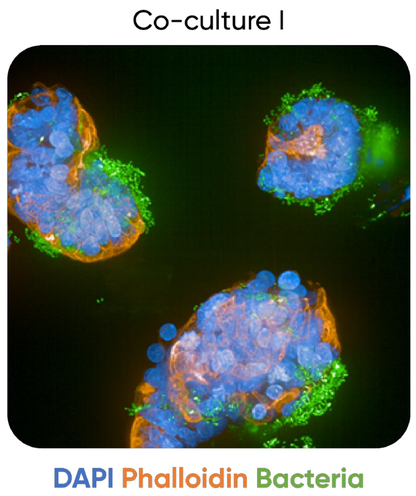
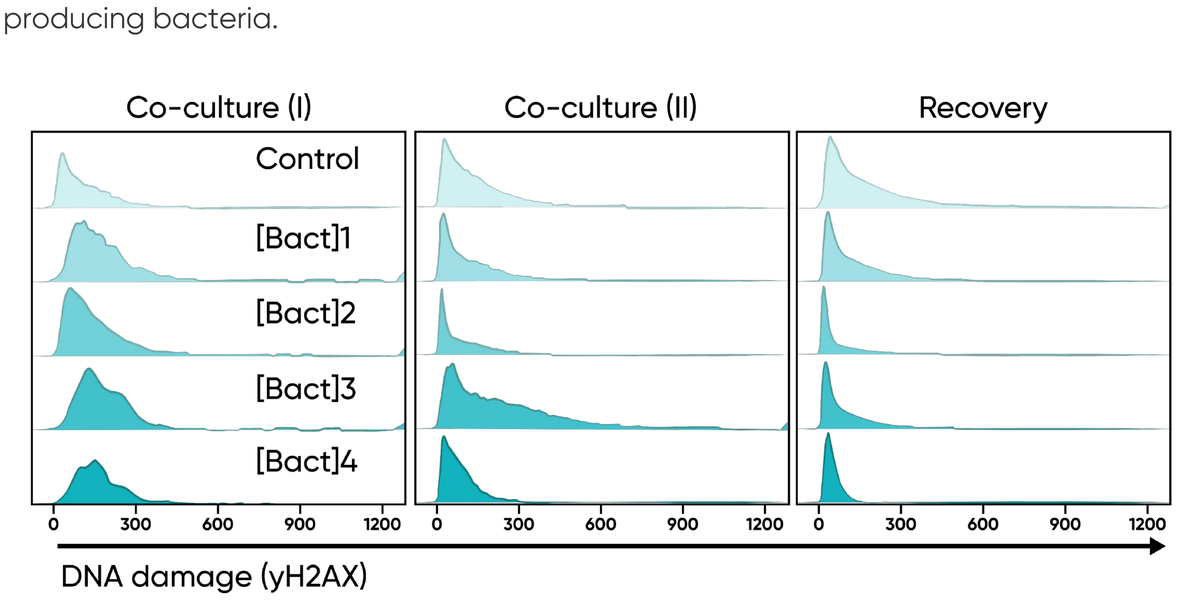


Fig 4. Colibacting-producting bacteria induces dna damage in the PDO fragment exposure model

Co-culture of PDOs fragments with *pks+ E. coli* (colibactin-producing bacteria) resulted in increased levels of yH2AX (proxy for DNA damage) in live single cells analyzed by flow cytometry. PDOs survive following co-culture with colibactin-producing bacteria.



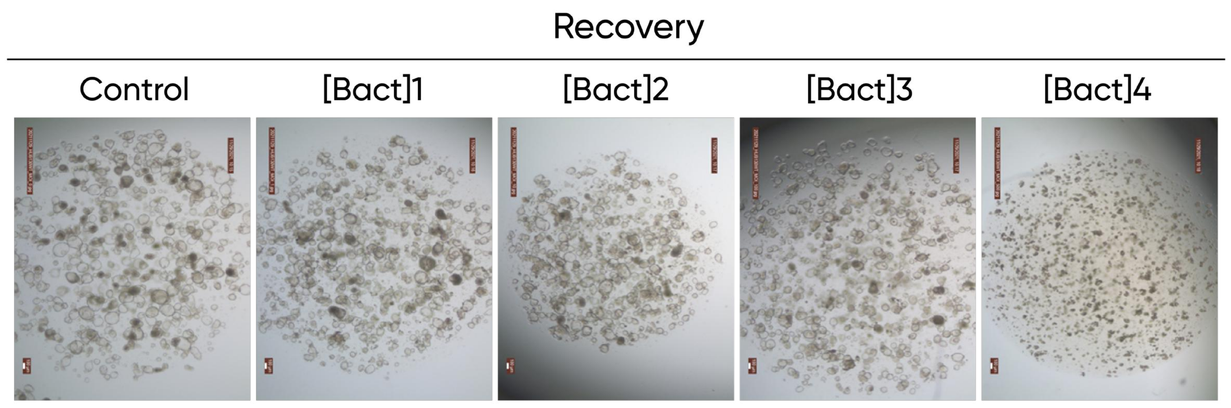
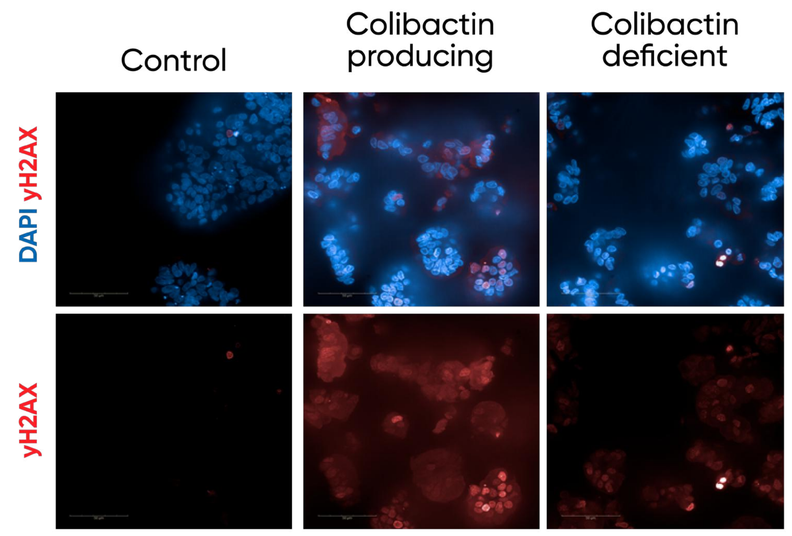
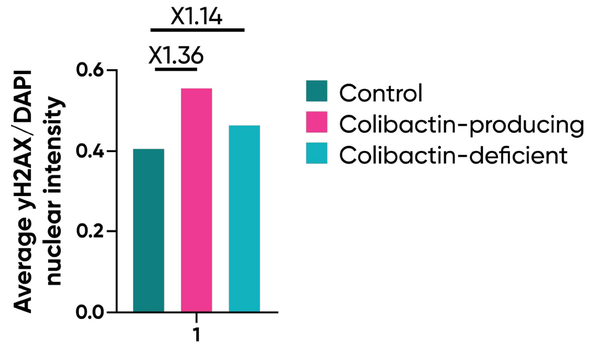


Fig 5. DNA damage detected in PDO fragment exposure model is dependent on colibactin production

PDO fragments were incubated with either colibactin-producing bacteria or an isogenic mutant strain lacking the machinery for colibactin production. Colibactin-producing bacteria induced higher levels of DNA damage (yH2AX) compared to colibactin-deficient bacteria, as determined by IF.





Conclusions

We have developed a PDO and bacteria co-culture system compatible with medium-to-high throughput screening readouts (IF, flow cytometry and comet chip). This co-culture system is currently tailored for the modeling of colibactin genotoxic effects in the gut epithelium but can be potentially extended as a discovery platform to identify targetable, novel, and complex interactions between host and pathogen.

References: [1]Pleguezuelos-Manzano *et al.* (2020)

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