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# Exploring the use of intestinal organoid models for advancing the application of New Approach Methodologies in food safety assessment

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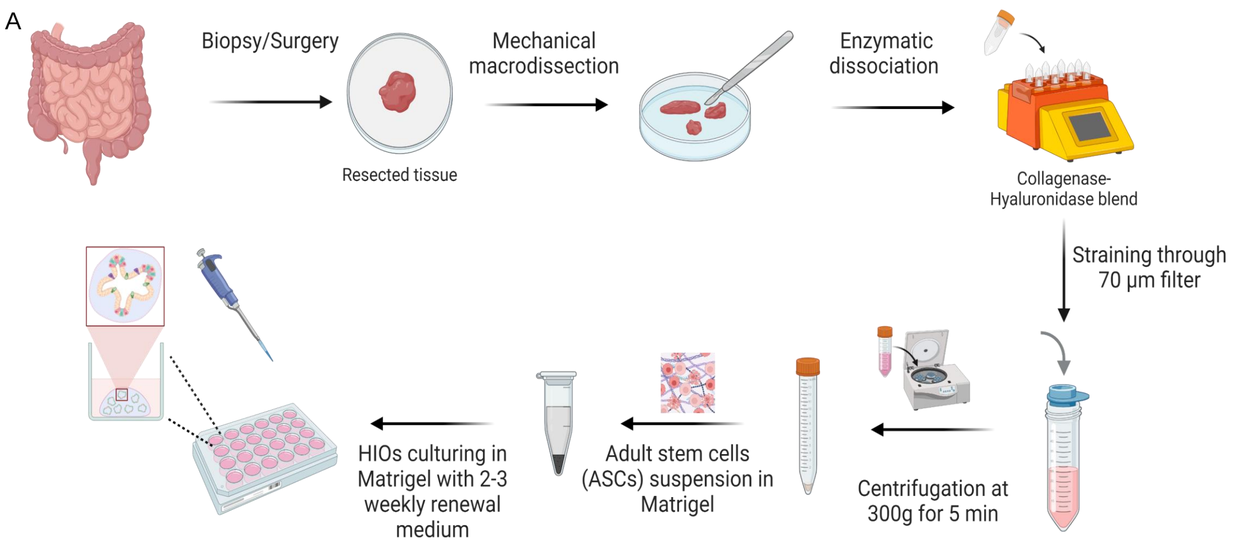
## INTRODUCTION & AIM

Regulatory toxicology and risk assessment are experiencing substantial changes. At a global level, institutions, regulatory agencies and the scientific community have embraced the objective of revolutionizing the paradigm of the evaluation of regulated products, contaminants and pollutants, with marked consequences for food safety standards. For these reasons, the conventional strategies that made massive use of animals have been gradually replaced by New Approach Methodologies (NAMs). These models, based on stand-alone or integrated *in vitro*, *in chemico*, *in silico* and *ex vivo* methods, guide the transition towards Next-Generation Risk Assessment (NGRA) [1,2]. The implementation and usage of NAMs in the regulatory context have been becoming main objectives of worldwide governmental bodies and agencies, including the European Food Safety Authority and United States Food and Drug Administration. Among NAMs, organoids are promising self-organized 3D in vitro models, able to mimic the key structural, functional and biological complexity of an organ and recapitulate physiology and molecular profiles closer to native tissue [3].

Looking at this context, this study aimed to set up and optimize an efficient methodology for the establishment of human intestinal derived organoids (HIOs) and verify that they faithfully express the molecular markers characteristic of intestinal cytotypes. The local effects of selected plant-based materials on the intestinal epithelium were assessed.

## METHODS



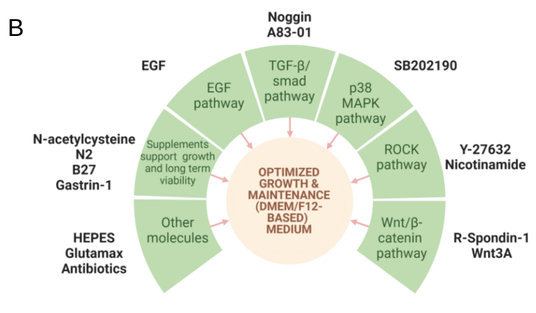
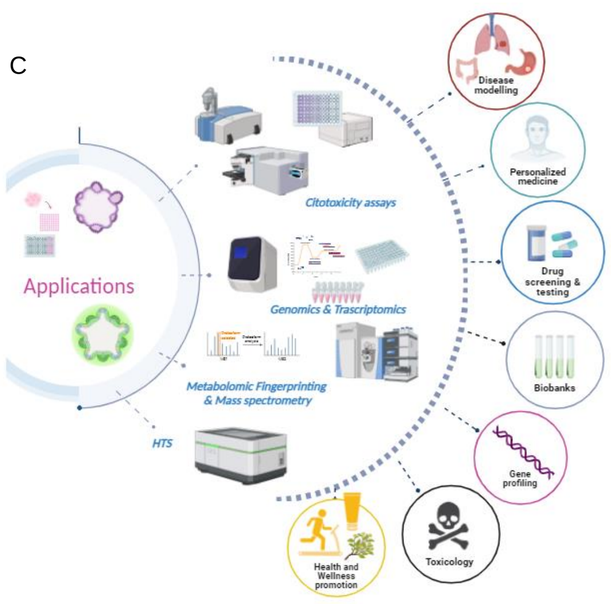
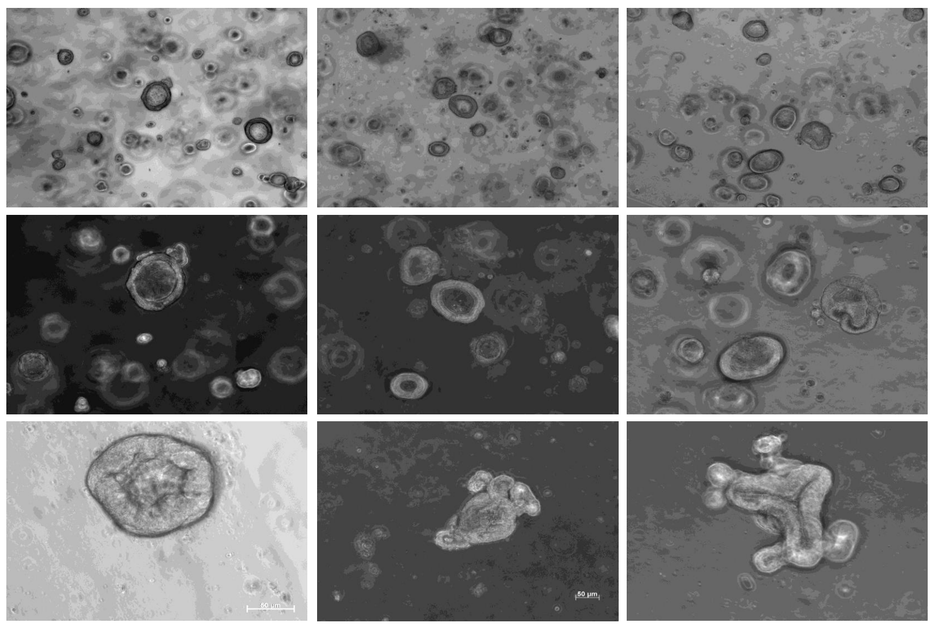




Fig.1 (A) Workflow for the establishment of HIOs. (B) Optimized growth and maintenance medium to ensure proper development and functionality of the organoids and involved pathways. (C) Key applications of HIOs are highlighted, such as their use in toxicological assessments, multi-omics studies, and fulfilling data requirements for regulatory purposes.




## RESULTS & DISCUSSION




| Markers | Intestinal Epithelial Cell | Localization | Role |
|---|---|---|---|
| Villin (VIL1), Intestinal alkaline phosphatase (Alpi) | Enterocyte | Small intestine (enterocyte)<br>Colon (colonocyte) | Physical barrier<br>Nutrient/water absorption<br>Epithelial shedding<br>Antimicrobial secretion |
| Mucin 2 (Muc2) | Goblet cell | Small intestine<br>Colon | Mucin secretion<br>Goblet cell-associated passage<br>Restitution |
| Lysozyme (Lyz) | Paneth cell | Small intestine | Antimicrobial secretion<br>Support the stem cell niche |
| DCLK1 | Tuft cell | Small intestine<br>Colon | Helminth detection<br>ILC2 expansion through<br>Production/secretion of IL-25 |
| Chromogranin A (ChGb) | Enteroendocrine cell | Small intestine<br>Colon | Hormones secretion |
| Glycoprotein 2 (GP2) | M cell | Small intestine (follicle-associated epithelium) | Antigen uptake |
| Leucine rich repeat containing G-protein-coupled receptor 5 (LGR5) | Intestinal stem cell | Bottom of intestinal crypt | Wnt signaling pathway<br>Stemness marker |

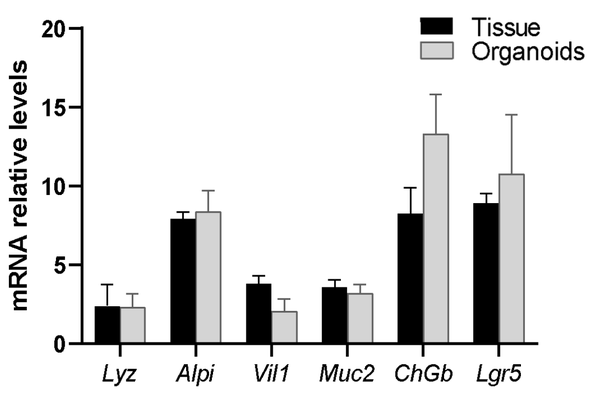



Fig.2 Assessment of model human relevance

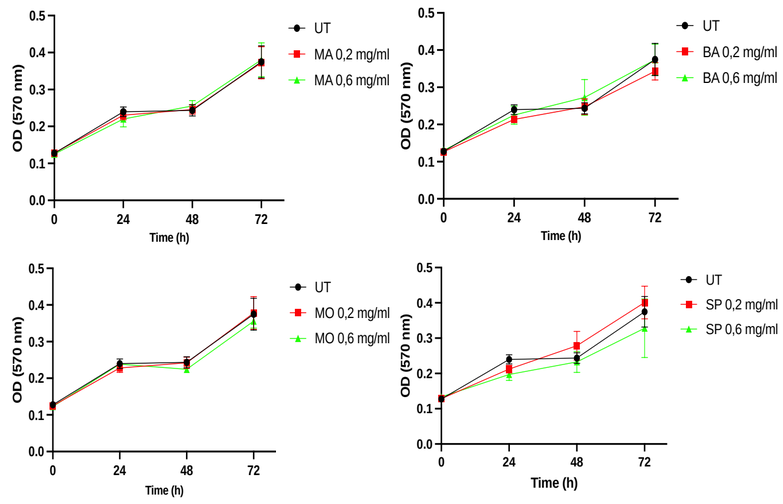
(A) Representative bright-field images of HIOs from three different donors and at different maturation stages (Day 4 to 6: first and second line; Day 10 to 14: third line). Scale bar: 50 µm.

(B) Schematic illustration and table of the intestinal crypt, highlighting the main intestinal cell types.

(C) Benchmarking HIOs as reproducible "replica'' of the intestinal epithelium: RT-qPCR analysis HIO/tissue markers. mRNA levels were normalized to GAPDH and actin expression. Data are presented as mean ± SD from five organoid samples or three tissue samples from different donors.

Fig.3 Safety assessment of food supplements: modified 3D-cytotoxicity assay (MTT)

Four dried powders intended for special nutrition, including *Lepidium meyenii* (Maca, MA) pulp, *Adansonia digitata* (Baobab, BA) root, *Arthrospira platensis* (Spirulina, SP) whole algae, and *Moringa oleifera* (Moringa, MO) leaves, were extracted and previously characterized by LC-MS/MS and ICP-MS. These extracts were then tested to assess their safety by evaluating the viability of human intestinal organoids (HIOs) at different concentrations and exposure times.




## ONGOING & FUTURE ACTIVITIES

- ✓ **Organoid Optimization and generation of pathological models**: Refining the methodology for consistent generation of high-quality HIOs and inflamed models.
- ✓ **Stress Testing Expansion**: Extending toxicant exposure studies to assess a wider range of contaminants and regulated products.
- ✓ **Molecular Characterization**: Continued validation of HIOs by examining additional intestinal markers and establishment of relevant molecular signature and cell types for greater precision.
- ✓ ***In-house* Validation Studies**: Conducting comprehensive *in-house* validation to standardize HIO protocols for regulatory use.
- ✓ **Collaboration with Regulatory Bodies**: Working with institution and agencies (e.g. EFSA and FDA) to integrate HIOs into regulatory frameworks.
- ✓ **Tissue-Specific Applications**: Developing and adapting organoid models for other tissues/organs to broaden applicability across various toxicology assessments.
- ✓ **Automated Organoid Production**: Exploring automation techniques to streamline HIO production for high-throughput screening in regulatory testing.

## CONCLUSIONS

This study successfully established and optimized a reliable methodology for generating HIOs that closely replicate native intestinal tissue. The molecular characterization confirmed that HIOs express key markers of intestinal cell types, validating their physiological relevance. HIOs demonstrated robust responses to selected products, making them promising candidates for regulatory toxicology and risk assessment applications. Moving forward, refining quality control measures and conducting thorough validation studies will be essential for integrating HIOs into regulatory frameworks, fulfilling global efforts to adopt NAMs. Overall, this research contributes to advancing non-animal testing models and paves the way for HIOs to be widely used in NGRA.

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