Dynamic *in vitro* models – the bridge between microtiter plates and animal studies

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Simulating the GIT

Human *in vivo* testing

Static *in vitro* models
+ low cost
+ not laborious
+ no ethical constrains
- low complexity
- no absorption
- no pH control

Animal *in vivo* models

Animal/human *in vivo* trials
+ high complexity
+ ileum microbiota
- ethical constrains
- high cost
- laborious
- high variation

Dynamic *in vitro* models
+ no ethical constrains
+ moderate complexity
+ pH control
- decreased throughput
Why do I need *in vitro* models?

- Decreased variability between samples
- Possibility to test different treatments on same individual
- Full control over experimental conditions and parameters
- Possibility to sample multiple times
- Focused on elucidating the mode of action
- Lower costs
- No ethical concerns
# Models existing on the market

<table>
<thead>
<tr>
<th>Name of the system</th>
<th>Volume [ml]</th>
<th>pH control</th>
<th>Maximal number of replicates</th>
<th>Intestine absorption simulation</th>
<th>Small intestine microbiota presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIME</td>
<td>300-1200</td>
<td>+</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TIM-1</td>
<td>300</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IViDiS</td>
<td>300</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**High costs of models and experiments**
TSI and CoMiniGut – characteristics

1. Low volume (up to 10 ml)
2. Increased throughput (5 samples per unit)
3. Simulate absorption of intestinal epithelium
4. Mimic small intestine microbiota
5. Simulate fasted and fed conditions
6. Simulation of microbial ecology of the colon
The Smallest Intestine *in vitro* model (TSI)
The Smallest Intestine

The Copenhagen MiniGut (CoMiniGut)
Main outcomes

Probiotic behaviour

SCFA production (metabolome)

Gut microbiota dynamics
Applications for TSI and CoMiniGut
Application 1: Testing survival of probiotic bacteria

"Live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host"
Application 2: Testing viability of microencapsulated bacteria

- Lactobacillus
- Streptococcus
- Leuconostoc
- A. muciniphila
- F. prausnitzii
- O. formigenes

Probiotics

Next-generation probiotics

Viability of microencapsulated *Akkermansia muciniphila* and *Lactobacillus plantarum* during freeze-drying, storage and *in vitro* simulated upper gastrointestinal tract passage.
Application 3: Testing persistence and performance of bacteriophages

Brief Report

A bacteriophage cocktail targeting *Escherichia coli* reduces *E. coli* in simulated gut conditions, while preserving a non-targeted representative commensal normal microbiota

Tomasz Cleplak, Nitzan Soffer, Alexander Sulakvelidze & Dennis Sandris Nielsen

Received 15 Sep 2017, Accepted 26 Feb 2018, Accepted author version posted online: 08 Mar 2018, Published online: 24 Aug 2018
Bacteriophage vs. Antibiotic

Fasted

Fed

Dias 13
Application 4: Testing survival and behaviour of *Bacillus* spp. spores in simulated piglet GIT

Spore structure

<table>
<thead>
<tr>
<th>Stress factor</th>
<th>Vegetative cells</th>
<th>Spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry heat, 105 °C</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2$ (15%)</td>
<td>&lt;0.2</td>
<td>50</td>
</tr>
<tr>
<td>UV-254 nm (KJ/m$^2$)</td>
<td>36</td>
<td>330</td>
</tr>
</tbody>
</table>
Application 5: Influence of pectin addition in pig feed on SCFA production and gut microbiota

CoMiniGut—a small volume *in vitro* colon model for the screening of gut microbial fermentation processes

Maria Wiese, Bekzod Khakimov, Sebastian Nielsen, Helena Sørensen, Frans van den Berg, Dennis Sandris Nielsen
A culture-independent method for studying transfer of IncI1 plasmids from wild-type *Escherichia coli* in complex microbial communities

Mehreen Anjum a, Jonas Stenløkke Madsen b, Carmen Espinosa-Gongora a, Bimal Jana a, Maria Wiese d, Dennis Sandris Nielsen d, Søren Johannes Sørensen b, Arshnee Moodley a, Valeria Bortolaia c, Luca Guardabassi a ∗