O1-4 **Probiosis and Bioencapsulation, where do they meet?**

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SUMMARY

The human large intestine is colonized by a dense and complex microbial community composed largely of anaerobic bacteria, whose cell numbers can exceed 10^{11} per gram. The activities of these organisms have a major impact upon nutrition and health of the human host by modifying nutrient supply, conversion of metabolites and/or interactions with host cells. For most practical purposes, however, the large bowel is inaccessible for routine investigation, and animal and *in vitro* model systems have been developed to study gut microbiota. Different *in vitro* models for intestinal fermentation, ranging from simple batch systems using pure or defined mixed populations of bacteria, or fecal material, to more sophisticated complex multistage continuous cultures have been used. *In vitro* models have no ethical guidelines and are well-suited for mechanistic studies. These systems are able to simulate many of the spatial, temporal and environmental attributes that characterize microbiological events in different regions of the large gut. They have limitations, however, due to the free-cell state of their bacterial populations and may not be fully representative of the complex bacterial community which is present both at planktonic and sessile states in the colon. Moreover, they often show much lower cell density compared with colonic contents as well as limited time stability.

We have developed a multiscale bioencapsulation approach combining *in vitro* colonic models and nanoparticule-based detection to study gut microbiota composition and activity and its response to environmental parameters and probiosis. Novel *in vitro* colonic fermentation models where the fecal microbiota are immobilized on a mixed polysaccharide gel bead structure selected for long term stability (chemical, biological and mechanical) during continuous colonic cultures in stirred tank bioreactors were set validated for different hosts and disease conditions. Infant, child and adult fecal microbiota were successfully immobilized using a two phase dispersion process carried out under strict anaerobiosis (Figure 1). After an initial colonization period of the beads, a stable continuous colonic culture working with environmental conditions akin to the gastrointestinal tract is achieved, while preserving major bacterial populations for up to 2 months.

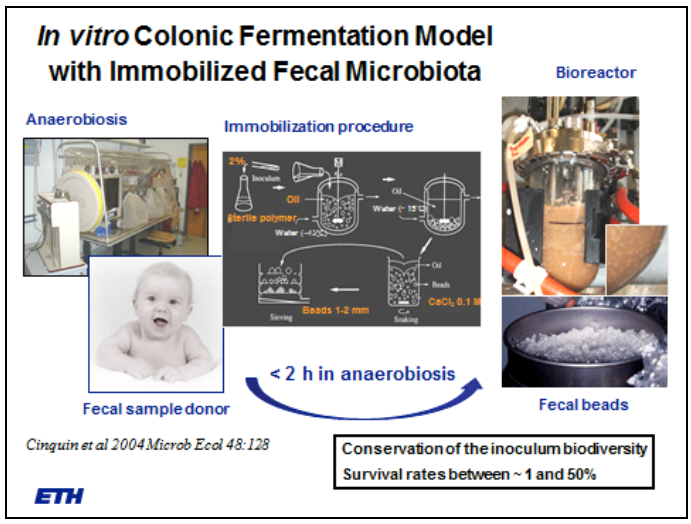

Figure 1. Immobilization of colonic microbiota in polysaccharide gel beads with a two phase dispersion system for intestinal fermentation models.

This technology was integrated in a three-stage chemostat simulating the conditions of the proximal, transverse and distal colons (Figure 2). The microbial community structure developed in the fermenter reflects the relative proportions and activities of the major bacterial groups present in fecal samples. These models have been successfully used to test the effects of different biotics (pre-, pro- and antibiotics) on gut microbiota and host interaction. Furthermore using the same approach we have recently developed colonic models for studying imbalanced or diseased gut microbiota (e.g. infected, anemic and obese subjects), leading to mechanistic studies and testing of preventive and therapeutic treatments. Colonic fermentation models were combined with human epithelial cell models to study *in vitro* microbial-host interactions (Figure 2). As example the mucus-secreting intestinal cell line HT29-MTX was chosen to test the effects of probiotics adhesion-invasion and competitive-exclusion of *Salmonella* in complex fermentation effluent and protection of cell junction integrity (transepithelial electrical resistance) and analyse inflammatory and anti-inflammatory cytokines secretion (Bioplex assay was applied with Transwell® co-culture system).

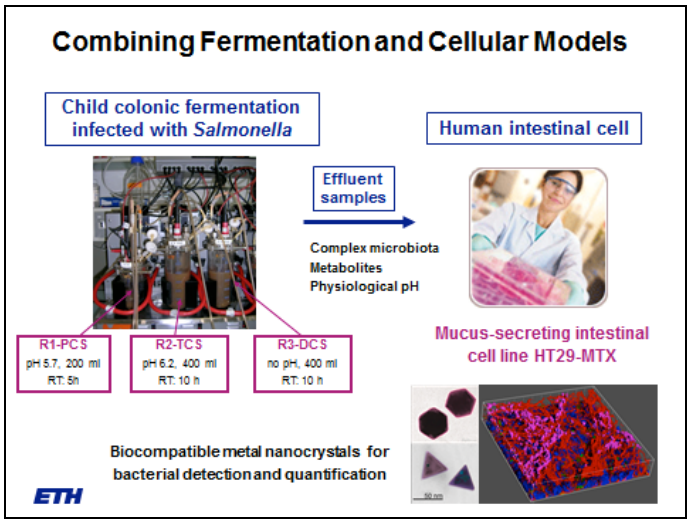

Figure 2. Colonic fermentation models were combined with human epithelial cell models to study *in vitro* microbial-host interactions

Finally functionalized metal nanoclusters produced with a novel synthesis protocol combined with Laser Scanning Confocal Microscopy were developed to detect and study interactions of specific microbial groups in gut tissues and assess microbial metabolic features in complex gut environments (Figure 3).

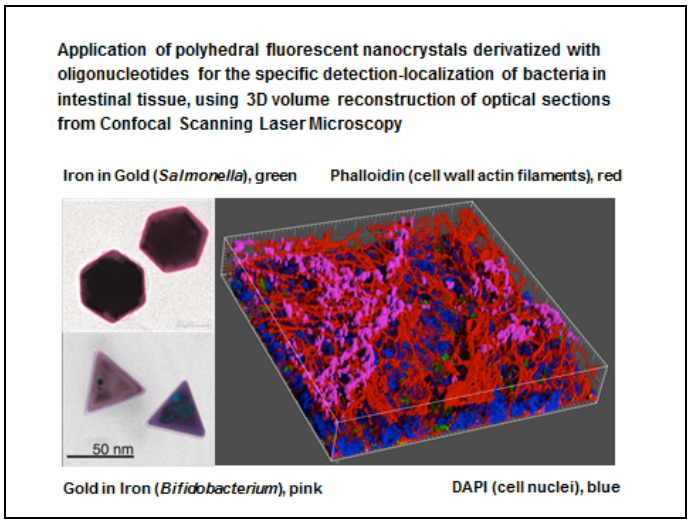

Figure 3. Targeted Nanocrystals for Specific Bacterial Detection-Localization in Intestinal Tissue

Combined multiscale bioencapsulation models can provide mechanistic information on gut microbiota composition and activities in healthy and disease subjects, unravel microbial metabolic functions in complex biota, and give crucial information on the metabolism of food compounds, drugs and toxic products.

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