Bioencapsulation and microbial transplant, what's the use?

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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^{14} (100 trillions) microbes belonging to > 1000 species, exceeding the total number of human cells by an order of magnitude. Presently, it is known that the metagenome of our intestinal microbes, also known as our microbiome, harbors over 3 million genes and vastly exceeds the coding capacity of our own genome. Indeed the distal gut microbiota activities may be considered as a distinct human organ responsible for multiple physiologic functions related to nutrition and health of the human host. Consequently there is considerable interest in understanding the effect of diet and host genes on the gut microbiota composition and activity. In addition, the relation between intestinal microbiota and disease is actively studied and over 25 diseases have been associated with our intestinal microbiome. These include intestinal disease including inflammatory bowel syndrome (IBS) and disease (IBD), and also more systemic diseases such as metabolic syndrome, type 1 and type 2 diabetes, obesity, and autoimmune, asthma, and allergic diseases, many of which have reached epidemic proportions in recent years.

Resident commensal bacteria are further responsible for creating a powerful line of resistance to colonization by exogenous microbes. Adherent intestinal microbes constantly compete with exogenous microbes for attachment sites in the brush border of intestinal epithelial cells, preventing pathogenic invasion and translocation into colonic tissue (Payne 2012). Colonization resistance requires a delicate ecological balance to avert the overgrowth of acquired opportunistic bacteria such as *Clostridium difficile* and prevent the pathogenesis of these strains. Optimized nutrient consumption and metabolism by resident gut microbiota further attenuates pathogen proliferation as resident microbes are capable of adjusting their nutrient requirements and metabolic activities causing pathogen starvation (Payne 2012).

One of the best examples of a disease resulting from major disruption of the gut microbiota by antibiotics is *C. difficile* infection (CDI). CDI has become a growing public health problem in the last two decades, with a high prevalence rate in acute care facilities, and accompanied by increasing rates of colectomy and death, with approximately 100,000 people dying annually in the U.S. with CDI. Older patients are particularly susceptible to CDI, but no age group is spared, and the incidence of CDI-related hospitalizations has been rising even in the pediatric population (Khoruts 2011).

Standard treatment of CDI is based on antibiotics, which have broad activity against the dominant phyla of colonic microbiota, but the risk of relapse following initial treatment of CDI is high approximately 20-25% (Khoruts 2011, Palmer 2011). Thus, a fraction of patients can develop chronic, recurrent form of CDI that can last indefinitely. It is now recognized that the presence of normal, healthy, intestinal microbiota offers protection against CDI.

Fecal microbiota transplantation (FMT), also commonly known as "fecal bacteriotherapy" represents the one therapeutic protocol that allows the fastest reconstitution of a normal composition of colon microbial communities (Palmer 2011). This rather simple therapeutic solution aims to re-establish a normal intestinal flora, deprived of *C. difficile*, by administration of fecal material obtained from a healthy donor into the colon of patients with the disease. For many decades, FMT has been offered by select centers across the world, typically as an option of last resort for patients with recurrent CDI. Well over 200 cases have been reported with an approximately 90 % cumulative success rate in clearing recurrent CDI, without any noted adverse events. The lack of wider practice of FMT is due to multiple nontrivial practical barriers and not due to lack of efficacy. Such barriers include the donor screening, the production of a repeatable suitable material, safety concerns and aesthetic reasons (Palmer 2011).

The design and complexity of *in vitro* fermentation systems has broadened from simple batch cultures to single- or multistage continuous flow models using a variety of different fecal inoculation techniques (Payne 2012). *In vitro* gut fermentation models enable the stable cultivation of a complete intestinal microbiota for a defined and model specific period of time. *In vitro* models have no ethical guidelines and are well-suited for mechanistic studies. Operation of most *in vitro* systems uses a liquid fecal suspension as inoculum, resulting in several limitations due to the free-cell state of the bacterial populations. Systems with liquid fecal inocula generally experience a rapid washout of less competitive bacteria and are consequently limited in operational time to less than 4 weeks (Payne 2012). These systems also struggle in reproducing both the planktonic (free-cell) and sessile (biofilm-associated) states of bacterial populations in the colon.

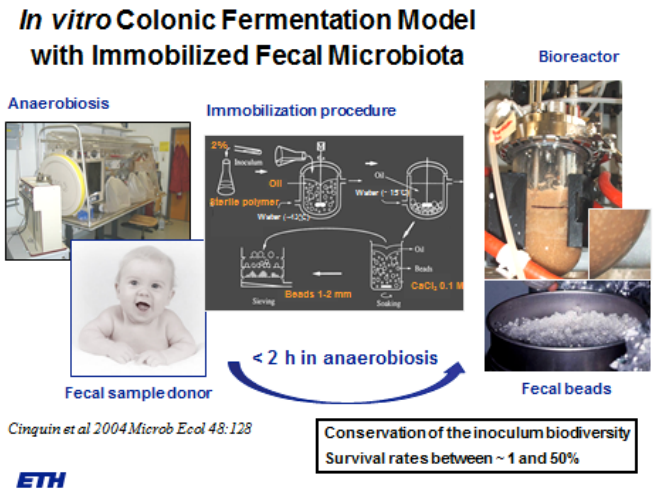

Figure 1. Immobilization of colonic microbiota in mixed gel polysaccharide gel beads with a two phase dispersion system for intestinal fermentation models, exemplified with baby fecal donor.

To address problems associated with inoculum washout, we developed a process for the immobilization of fecal microbiota (Figure 1) which was applied in a variety of fermentation designs to stably cultivate and study the gut microbiota of different hosts (from infant to elderly), diet and health conditions (e.g. infections), while preserving the initial biodiversity of the donor fecal sample (Payne 2012). Here, fecal microbiota are suspended within a porous polysaccharide matrix resulting in the formation of fecal beads, which are transferred to the growth medium in R1 of a multistage continuous fermentation model (Figure 2). Limitations on substrate and toxic product diffusion within beads result in formation of a high-cell density peripheral layer, where cell release occurs spontaneously as a result of active cell growth. The released cells are transported to R2 and then R3, resulting in a self-contained continuous fermentation system of very high-cell density and population stability, close to the human GI tract (Figure 2).

The operational time of systems for studies using immobilized cells varies and has been functionally demonstrated for more than 3 months. We also showed using both cultivation and advanced molecular methods for population profiling (quantitative real-time PCR, TGGE, HITChips, pyrosequencing) that the microbial community structure developed in the fermenter reflects the relative proportions and activities of the major bacterial groups present in fecal samples. Moreover, the biodiversity indices of fecal inocula and effluent samples from the distal colon reactor (most akin to feces) are very similar, exemplifying the preservation of the complex inoculum diversity. Inoculation and colonization of the system, however, results in a newly balanced gut microbiota which is a result of both environmental factors and the initial qualitative diversity, but not initial quantitative balance of the fecal inoculum. These changes in population ratios reflect applied fermentation conditions (e.g. retention time, culture medium, pH, etc.) which can never be an absolute simulation of the conditions encountered in the host intestine and the inability to simulate major host functions. Because environmental factors can be manipulated *in vitro*, the enhancement of beneficial components of the gut microbiota can be envisaged, for example by supplying selective nutrients or changing the pH.

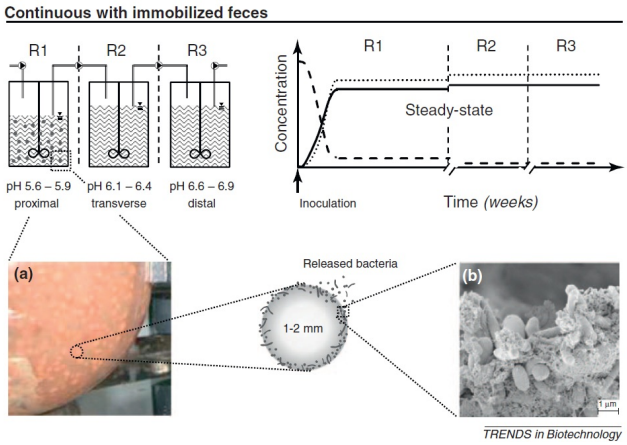

Figure 2. In vitro colonic fermentation models, inoculated with fecal microbiota immobilized in gel beads, simulating proximal (R1), transverse (R2) and distal (R3) colon regions. Initial (R1) and steady-state (R1, R2 and R3) fermentation profiles. (a) Picture detail of a proximal colon reactor containing polysaccharide beads with immobilized fecal microbiota. (b) Electron microscope image of microbes embedded and attached to the surface of an intestinal bead (Payne 2012).

CONCLUSION

Based on the excellent characteristics of our intestinal fermentation models with immobilized feacl microbiota, we suggest that such systems can be used to stably cultivate healthy complete gut microbial for application as high quality and safe control inocula for FMT. Furthermore the downstream processing, including formulation of a protective phase, stabilization and storage conditions for complex anaerobic gut microbiota, must be designed.

REFERENCES

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