Modulation of gut microbiome in prevention and treatment of chronic diseases

• RNDr. Jana Štofilová, PhD.

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Overview

Institute of Experimental Medicine
- Research team
- Infrastructure
- Long-term research program

The role of gut microbiota in health and disease
- Factors affecting the composition of gut microbiota

Modulation of gut microbiota in diseases

Probiotics and prebiotics

Fecal microbiota transplantation

Project APVV TRANSMICROBIOM
GROUP LEADER:
Alojz BOMBA, DVM, DSc

- 6 senior scientists
- 5 junior scientist
- 3 PhD students
- 3 technicians
Institute of Experimental Medicine

**Key questions:**

What is the role of gut microbiome in pathogenesis of chronic diseases?

What are possibilities for prevention of chronic diseases using targeted modulation of gut microbiome?
OUR EXPERTISE

METHODS

MODELS

*In vivo* rat models (colitis, cancer, dysbiosis)

*In vitro* model of human GIT (SHIME®)

Cell culture models

ANALYSIS

MICROBIOME (PCR-DGGE, qPCR, NGS)

SERUM PARAMETERS

GAS CHROMATOGRAPHY

FLOW CYTOMETRY
Infrastructure
Infrastructure

Molecular biology Lab

Microbiology Lab

Cell culture Lab

Biochemistry Lab

Mass Spectrometry Lab

Immunology Lab
TWINSHIME®
Simulator of Human Intestinal Microbial Ecosystem
The 40th International Congress of the Society for Microbial Ecology and Diseases (SOMED 2018, Hungary)


International Scientific Conference of Society for Microbial Ecology and Disease (SOMED 2013, Slovakia)

European Researchers' Night
The overall mission of the HMP was to generate resources to facilitate characterization of the human microbiota to further our understanding of how the microbiome impacts human health and disease.

The initial phase of the project, HMP1, established in 2008, characterized the microbial communities from 300 healthy individuals, across several different sites on the human body: nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract, using 16S and metagenomic shotgun sequencing.

The second phase of the HMP (iHMP, Integrative Human Microbiome Project, 2013–2016) examined the role of the microbiome in human health and disease through a study of three models of microbiome-related human conditions (Pregnancy & Preterm Birth, IBD and type 2 diabetes).

https://hmpdacc.org/
Human Microbiome

- collection of all the microorganisms living in association with the human body
- eukaryotes, archaea, bacteria and viruses
- 500 – 1000 different species
- **10x more of bacteria** than human cells
- **1000 times more microbial genes** than are found in the entire human genome
- **0.9-2.7 kg bacteria in 90kg human**
- microbes are essential for maintaining health
- scientific exploration of the microbiome is in it’s infancy
Gut Microbiome

Density
$10^1 - 10^2$
$10^3 - 10^4$
$10^4 - 10^7$
$10^{10} - 10^{13}$

Composition
Streptococcus
Lactobacillus

$10^4 - 10^7$
Streptococcus
Lactobacillus
Enterobacteriaceae

$10^{10} - 10^{13}$
Bacteroides
Eubacterium
Clostridium
Ruminococcus
Bifidobacterium

Intrinsic factors
Gastric acid
O$_2$
Motility
Mucus
GI secretions
Antimicrobial peptides
Immunity (sIgA)

Extrinsic factors
Diet, Pre and probiotics
PPIs, H2 blockers
Antibiotics
Prokinetics
Laxatives
Opioids
NSAIDs

MMC
H$^+$

colon contractions

O$_2$

Stomach
Small intestine
(facultative anaerobes)
Colon
(strict anaerobes)
Functions of gut microbiota

1. Protection against pathogens
2. Synthesis of vitamins (K, B12)
3. Immune system development
4. Promotion of intestinal angiogenesis
5. Promotion of fat storage
6. SCFA production by fermentation of dietary fiber
7. Modulation of central nervous system
Factors affecting the composition of gut microbiome
Birth mode and infant feeding method

- Vaginally born - ↑ *Lactobacillus, Prevotella* coming from maternal vaginal tract
- C-section - ↑ *Staphylococcus, Corynebacterium, Propionobacterium, Clostridium*
- Breast feeding – dominance of *Bifidobacterium*
- Formula feeding - ↑ diversity of bacteria
Diet

- diet is an important driver of microbiome composition in humans
- gut microbiota composition differs according to diet and eating habits
- omnivorous group has a higher diversity of bacteria compared to vegetarians

- Comparison of the intestinal microbiota of children from Africa - Burkina Faso (BF) with the microbiota of children in the EU

- Diet rich in fiber and indigestible polysaccharides leads to ↑ *Bacteroides* against *Firmicutes* in BF children

Diet

Low-fat, high-fiber diets

High-fat, low-fiber diets

Intestinal microbiota diversity

Short chain fatty acids

Gut homeostasis

↑

↑

↓

↓

↓
Enterotypes of gut microbiome

- Enterotypes are clusters of bacteria that dominate in a person’s microbiome.
- Clusters are associated with specific long-term eating patterns.
- The phylogenetic profile of each individual can be categorized into 3 enterotypes dominated by different metabolic pathways:
  - **Enterotype 1** – *Bacterioides*
  - **Enterotype 2** – *Prevotella*
  - **Enterotype 3** – *Ruminococcus*
- Age, gender and body mass don't appear to influence enterotype

**High Fiber Diet**

- **Bacteroidetes**
  - Bacteroides-Prevotella spp.
  - Firmicutes
    - Bacilli
    - Bifidobacterium
  - Clostridia group
  - Ruminococcus
  - Lactobacillus-Enterococcus group
  - R. prausnitzii and E. rectale-C. cocoides groups
- Actinobacteria
- Bifidobacterium
- Proteobacteria
- Desulfovibrio

**Bacteroidetes**

- Bacteroides enterotype
- Prevotella enterotype
- Lactobacillus
- Bifidobacterium
- Rikenella
- Peptostreptococcus
- Bacteroides enterotype
- Proteobacteria
- Desulfovibrio
- Defembacteres
  - Mucispirillum
  - Firmicutes
  - Clostridium genus

**High Protein Diet**

- **Bacteroidetes**
  - Bacteroides enterotype
  - Prevotella enterotype
  - Lactobacillus
  - Bifidobacterium
  - Rikenella
  - Peptostreptococcus
  - Bacteroides enterotype
  - Proteobacteria
  - Desulfovibrio
  - Defembacteres
  - Mucispirillum
  - Firmicutes
  - Clostridium genus

**High Fat Diet**

- **Bacteroidetes**
  - Rikenellaceae
  - Bacteroides spp.
  - Bacteroides enterotype
  - Firmicutes
  - Eubacterium rectale
  - Blautia cocoides
  - Ruminococcaceae
  - Bacilli
  - Bifidobacterium
  - Roseburia spp.
  - Proteobacteria
  - Desulfovibrio

**Carbohydrates Diet**

- **Bacteroidetes**
  - Bacteroides enterotype
  - Firmicutes
  - Prevotella enterotype
  - Roseburia groups
  - Bifidobacteria
  - E. rectale
  - Ruminococcus bromii
  - R. flavefaciens
  - R. albus
  - Actinobacteria
  - Bifidobacterium
Ageing

- onset and shaping of human gut microbiota through life stages and perturbations
- babies have low diversity of the microbiota
- the microbiota of 2.5 year olds is already similar to that of adults
- the microbiota of adults is stable
- with the age the diversity of microbiota declines (↓ diversity and metabolic activity – SCFA, ↓ immune system)
• The taxonomic composition of the gut microbiome associates with patient ethnicity and geographic location.

• Certain taxonomic groups of bacteria are a characteristic feature of a given geographical area irrespective of the diet or age of the population.
Exercise

- Physical exercise is able to modulate gut microbiota and increase the abundance of beneficial microbial species.
- Increasing physical activity in obese animals lead to the changes in gut microbiota composition connected with weight lose and lipid metabolism modulation.

Carbajo-Pescador et al. 2019, Disease Models & Mechanisms 12, dmm039206
Changes in the composition and functions of our microbiomes (dysbiosis) correlate with numerous disease states, raising the possibility that manipulation of these communities could be used to treat disease.
Gut Homeostasis

- Commensal Bacteria
- Mucus
- Functional Barrier
- Tolerant Immune Response

Dysbiosis

- Microbial Dysbiosis
- Barrier Defect
- Dysregulated Immune Response

PRRs: Pattern Recognition Receptors
IgA: Immunoglobulin A
Dysbiosis associated diseases
Gut dysbiosis-associated diseases

Alteration of gut microbiota

**Psychiatric disorders**
- autism
- depressive disorders
- Alzheimer disease

**Infectious diseases**
- *Clostridium difficile*
- *Helicobacter pylori*
- bacterial vaginosis
- Infection with HIV

**Liver diseases**
- acute/chronic liver failure
- nonalcoholic steatohepatitis
- Nonalcoholic fatty liver disease

**Metabolic disorders**
- obesity
- type 2 diabetes

**Cancer**
- gastric cancer
- colorectal cancer
- esophageal cancer
- breast cancer
- prostate cancer
- liver cancer

**Inflammatory bowel diseases**
- Crohn disease
- ulcerative colitis
- irritable bowel syndrome

**Allergic disease**
- asthma
- food allergies
- celiac

**Psychiatric disorders**
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- celiac

Gut microbiota of healthy, lean vs. obese human
Manipulation of gut microbiome

Direct strategies

Indirect strategies

Probiotics are defined as living bacteria that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2001).
Desirable selection criteria for potential probiotic microorganisms

Safety criteria
- Human origin
- Non-pathogenic
- Resistance to antibiotics

Functional criteria
- Resistance against acid and bile condition
- Adhesion to mucosal surface
- Clinically validated and documented health effects

Technological criteria
- Genetically stable
- Good sensory properties
- Phage resistance
- Large-scale production
- Desired viability during processing and storage

Physiological criteria
- Antagonism against enteric pathogens
- Lactose tolerance
- Cholesterol assimilation
- Anticarcinogenic and mutagenic properties
- Immunomodulation

Selection criteria for potential probiotics
Probiotics

- Conventional Probiotics
- Potentiated Probiotics and Synbiotics
- Engineered Probiotics
- Autoprobiotics
- Next Generation Probiotics
**Conventional probiotics**

### Table

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Strain</th>
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</table>
| Lactic Acid Bacteria | *Lactobacillus rhamnosus GG*  
|                   | *Lactobacillus casei*  
|                   | *Lactobacillus casei Shirota*  
|                   | *Lactobacillus acidophilus*  
|                   | *Lactobacillus johnsonii*  |
| Bifidobacteria    | *Bifidobacterium breve*  
|                   | *Bifidobacterium bifidum*  
|                   | *Bifidobacterium infantis*  
|                   | *Bifidobacterium animalis*  |
| Yeasts            | *Saccharomyces cerevisae boulardii*  |

*The effect is strain specific!!!*
Prebiotic

• a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host.

• Synbiotic
Potentiated probiotics

- Improvement of the probiotic effect of microorganisms by their combination with specific and non-specific substrates = synbiotics
- Enhancement of the probiotic effect of microorganisms by their combination with plants,

The application of probiotics and flaxseed promotes metabolism of n-3 polyunsaturated fatty acids in pigs

Drahomíra Sopková, Zdenka Hertelová, Zuzana Andrejčáková, Radoslava Vlková, Soňa Gancarčíková, Vladimir Petrill, Silvia Ondrášcová and Lenka Krešáková

Department of Anatomy, Histology and Physiology, University of Veterinary Medicine in Košice, Košice, Slovak Republic; Institute of Experimental Medicine, University of Pavol Jozef Safarik University in Košice, Košice, Slovak Republic; Institute of Gastroenterology and Microbiology, University of Veterinary Medicine in Košice, Košice, Slovak Republic.
Engineered probiotics

- improve stress tolerance
- antimicrobial and antiviral action
- toxin neutralization
- prevention of colonization
- regulation of virulence gene expression
- production of antimicrobial factors
- immunomodulation and cytoprotection
Autoprobiotics

- Autoprobiotic technology is based on the indigenous bacteria used for restoring the normal microbiota in the case of a dysbiotic condition.
• Gut microbiota is a source of novel health-promoting bacteria, often termed as next-generation probiotics in order to distinguish them from traditional probiotics
• They do not have a long history of safe use and their safety is not thus considered as proven
• Live microorganisms identified on the basis of comparative microbiota analyses between both healthy and unhealthy individuals
Postbiotics

- non-viable bacterial products or metabolic products from microorganisms that have biologic activity in the host
Antibiotic treatment alters the population structure of the indigenous microbiota, reducing bacterial diversity and redistributing member composition in both transient and persistent effects.
• FMT comprises the administration of a fecal solution from a donor into the intestinal tract of a recipient
How Fecal Transplantation Works

1. In a fecal transplant, stool from a healthy donor is used to replace a patient's gut microbial flora.

2. Exact preparations vary, but usually the stool is blended with saline and put through a strainer. It can be frozen before use.

3. The stool can be applied into the small intestine via a tube through the nose or mouth (a) or deep into the colon, using a colonoscopy (b). Enemas are popular for at-home treatments, but they only reach the lower end of the colon (c).

4. In the future, scientists hope to replace fecal transplants with an odorless mix of bacterial strains derived from human stool, grown in the lab. It could be applied using existing methods or in capsules.
Donor selection of fecal sample

- someone who is **healthy and on no medications**
- use same **exclusions as for blood product donation** (travel history, sexual behavior, previous operations, blood transfusions, etc...)
- screen donor for a **family history of autoimmune and metabolic diseases, malignancies**
- Screen blood and fecal samples for:
  
  ![Table 1. Amsterdam Protocol for FMT via Gastroduodenoscopy](#)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Screening for transmittable diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fecal pathogens: bacteria (<em>Helicobacter pylori</em> antigen, <em>Yersinia</em>, <em>Campylobacter</em>, <em>Shigella</em>, <em>Salmonella</em>, enteropathogenic <em>E. coli</em>), viruses (rotavirus, adenovirus, enterovirus, parechovirus, sapovirus, norovirus, and astrovirus), and parasites (triple feces test for ova and parasites, <em>Giardia</em>)</td>
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<table>
<thead>
<tr>
<th>Screening for other criteria</th>
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<tbody>
<tr>
<td>Diarrhea</td>
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<tr>
<td>Recent use of medications (within 3 months, mainly antibiotics or proton pump inhibitors)</td>
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<tr>
<td>Risk factors for transmittable diseases</td>
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<tr>
<td>Abnormal defecation patterns and symptoms of irritable bowel syndrome</td>
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<thead>
<tr>
<th>Feces</th>
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<tr>
<td>Freshly produced (within 6 hours of treatment)</td>
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<tr>
<td>At least 150 g (directly covered in 500 mL sterile saline 0.9% solution), subsequently filtered for a homogeneous solution</td>
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<tr>
<th>Patient</th>
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<tr>
<td>Placement of duodenal tube and small intestinal biopsies</td>
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<tr>
<td>Bowel lavage with 1–2 L of macrogl through duodenal tube</td>
</tr>
<tr>
<td>Administration of fecal solution through duodenal tube</td>
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<tr>
<td>No antibiotics before procedure</td>
</tr>
</tbody>
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Smith LP et al. Gastroenterology 2013;145:946-953
Preparation of FMT material

- Basic protocol:

  1. 50-300 g of feces are collected from the donor.

  2. Feces are dissolved in 50-100 ml of normal saline.

  3. Fecal materials are filtered through a metal strainer.

  4. Fecal slurry is administered through colonoscopy.
Routes of administration

- nasogastric tube
- nasojejunal tube
- upper tract endoscopy (esophagogastroduodenoscopy)
- colonoscopy
- retention enema
- oral capsules
- the best route most likely depends on the anatomic location of the disease
Therapeutic potential of FMT
• The Microbiome Health Research Institute, d.b.a. OpenBiome, nonprofit organization dedicated to expanding safe access to fecal microbiota transplants (FMT), and to catalyzing research into the human microbiome.

• Founded by a team of doctors, scientists and public health advocates, OpenBiome has two primary objectives:
  1. to eliminate the practical barriers to fecal microbiota transplantation
  2. to enable translational research into the human microbiome

https://www.openbiome.org/home/
Targeted modulation of gut microbiota and its transplantation in prevention and treatment of inflammatory bowel diseases
Institute of Experimental Medicine FM PJŠU in Košice

1st Department of Internal Medicine, FM PJŠU and Louis Pasteur University Hospital in Košice

Institute of Biology and Ecology, Faculty of Science PJŠU

Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice

ProDigest, Belgium – technical & methodological support

TEKMAR Slovakia, Ltd.

Monsea, Ltd.
Goals & Objectives of project

1. Clarification of composition, diversity and functions of the healthy people’ and IBD patients’ gut microbiota

2. Study of the effect of faecal microbiota transplantation (healthy donor to recipient with IBD) on the composition and functions of the target gut microbiota using SHIME

3. Study the possibilities of targeted modulation of the microbiota in patients with IBD by its modification using SHIME and its reverse transfer

4. *In vivo* verification of the FMT and SHIME modulated IBD microbiota effectiveness in animal models (gnotobiotic mice associated with human microbiota and conventional rats)
THE PROJECT WORKFLOW

**GUT MICROBIOME CHARACTERIZATION**
Healthy & UC patients

**IN VITRO EXPERIMENTS**
GIT models, cell culture models, anaerobic microbiology
Gut microbiome simulation, *in vitro* modulation of UC dysbiosis, *in vitro* FMT testing

**IN VIVO VERIFICATION**
Rat / mouse UC models
FMT method verification
1st phase

• Collection of samples (feces, blood)

• Study of the composition, function and diversity of the intestinal microbiota of healthy people and IBD patients
  • Molecular, microbiological and biochemical analyses

• Testing of various microbiota biomodulators (probiotic bacteria or natural bioactive substances) which could affect epithelial barrier integrity and immune functions in vitro
  • *L. plantarum LS07, L. reuteri, prebiotics, PUFA, etc.*
Microbial analyses of samples based on molecular methods

- isolation gDNA from stool samples
- qualitative characterization of microbiota composition (PCR-DGGE) Euubacteria, Bacteroidetes, Lactobacillus and Clostridium(Blautia) coccoide groups

- quantitative analyses of microbiome by real time qPCR
  - no significant differences in Eubacteria and Bacteroidetes

- NGS 16S rRNA sequencing (in progress)
<table>
<thead>
<tr>
<th>Eubacteria</th>
<th>Healthy</th>
<th>Colitis</th>
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<tbody>
<tr>
<td>Bacteroides fragilis</td>
<td></td>
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<tr>
<td>Lactobacillus plantarum</td>
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<td>Staphylococcus aureus</td>
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<tr>
<td>Enterococcus faecalis</td>
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<tr>
<td>Escherichia coli</td>
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<table>
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<tr>
<th>Bacteroidetes</th>
<th>Healthy</th>
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**Diagram A**

**Diagram B**
**Blautia coccoides group**

A

Bacterial species

- Healthy
- Colitis

**Lactobacillus group**

B

- Bacteroides fragilis
- Lactobacillus plantarum
- Staphylococcus aureus
- Enterococcus faecalis
Metabolic activity of gut microbiota

Microbial enzymatic analyses (fresh stool samples)
Spectrophotometric analyses of enzyme level:
- β-glucuronidase
- β-glucosidase
- β-galactosidase
- α-galactosidase
- α-glucosidase

Biochemical analyses of organic acids in blood serum
- determination of short-chain fatty acids levels (acetic, propionic, butyric, valeric acid, and isovaleric acid, caproic acid and isocaproic) by gas chromatography with flame ionization detector and mass-spectrophotometry.
Testing of various microbiota biomodulators (probiotic bacteria or natural bioactive substances)

• which could affect epithelial barrier integrity and its function
• inhibit the pathogen adherence
• which could have immunomodulatory effect on M1 and M2 macrophages and PBMC
Gut barrier and microbiome
In vitro models of gut barrier based on the immortalised epithelial cell lines cultivation
Measurement of transepithelial electrical resistance (TEER) of cells growing on a microporous membrane
xCELLigence SP RTCA system for real time monitoring the intestinal barrier function
Effect of lactobacilli and inulin on gut barrier

![Graph showing the effect of lactobacilli and inulin on gut barrier]

- Lactobacillus plantarum LS 07
- Lactobacillus reuteri
- Lactobacillus plantarum LS 07 + inulin
- Lactobacillus reuteri + inulin

Normalized Cell Index vs. Time (in Hour)
Immunomodulatory effect of probiotics and natural substances

- **THP-1 monocytic line** – differentiation on M1 or M2 macrophages, phagocytic activity, cytokine production after 24h bacterial stimulation
- **Peripheral blood mononuclear cells** isolated from healthy human and patients with UC – cytokine production after stimulation with bacteria
2nd phase:

- Study of the effect of healthy donors FMT on the IBD patients’ microbiota composition and functionality using *in vitro* TWINSHIME
3rd phase:

- Detection of optimal biomodulators for targeted modulation of IBD microbiota using *in vitro* TWINSHIME
- Return transfer of the IBD patients’ modulated microbiota to native IBD microbiota using *in vitro* TWINSHIME

**Modulation of microbiota by supplementation with probiotic strains, prebiotics, PUFA, etc.**

**Microbiota Transfer**

**Inoculation**

**Patient with UC** — **Patient with UC**

**Sample collection**

**Molecular analysis of gut microbiota composition (DGGE, qPCR)**

**Biochemical and metabolic analysis of microbiota activity**
4th phase:

- *In vivo* verification of the FMT and SHIME modulated IBD microbiota effectiveness in animal models (gnotobiotic mice associated with human microbiota and conventional rats)
  - UC chemicaly-induced by DSS
  - analyses of changes in gut microenvironment (microbiological, biochemical, physiological parameters of the metabolism and utilization of nutrients) & morphological and immunological parameters
• Original solution of gut microbiota modulation which could possibly meet criteria of the **personalized medicine approach**

• Our solution **eliminates the risks** connected with the fecal microbiota transplantation from donor and allows **targeted modulation according to specific needs of the patient**
Thank you for your attention.