Microbial ecosystem therapeutics - a new paradigm in medicine

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Presenter Disclosure

• I have relationships with commercial interests: NuBiyota LLC
Human health depends on microbiota health

We are super-organisms of human and microbial cells
We exist in a delicate host: microbe equilibrium
There are more bacteria living in your gut than there are people on the planet...

The Continent of Gut: 200-500 bacterial species

Also Archaea as well as yeasts & other microscopic eukaryotes
Everyone is different

Gut microbial ecosystems are highly variable in composition and abundance profiles between people.
Balance is important

High diversity of species:
• Healthy ecosystem
• Balance
• Functional redundancy
• Resistance to disease

Low diversity of species:
• Sick ecosystem
• Imbalance
• Functional disability
• Susceptibility to disease
Remarkably...

The bacterial community in your gut remains stable from

- weaning...
- ...to old age

And we are only just starting to understand this homeostasis
How do we acquire our gut microflora?

‘sterile’ at birth

Breast milk contains beneficial microbes

…and our environments

We obtain microbes from our food

The ‘window’ for gut microbial establishment is narrow
What do our gut microbes do for us?

**Metabolic**
- Fermentation of non-digestible substrates
- Production of vitamins, SCFA
- Removal of toxins, carcinogens
- Differentiation of IECs

**Structural**
- Intestinal villi and crypts
- Tight junctions
- sIgA production
- Mucus secretion

**Protective**
- Colonization resistance
- Innate and adaptive immunity
- Inflammatory cytokine oversight

**Energy**
- Competition for sites and nutrients
- Immune system and barrier function

After Cryan *et al.*
Our microbes are vitally important…

• But we are working very hard to exterminate them!
‘Extinction events’ may impact health

• Hygiene hypothesis
  – We are preventing proper colonization by being too clean

• Missing microbiota hypothesis
  – We are disturbing proper colonization across generations through e.g. antibiotic use

• Antibiotic use (especially in early childhood) may be particularly problematic
Many studies have shown:

– Gut microbiota changes significantly with antibiotic use
– Takes a long time afterwards to return to baseline
– Sometimes does not return to baseline at all
– Repeated ‘hits’ cause vast changes from which the ecosystem does not recover

The average person in the U.S. will receive 10-20 courses of antibiotics by the time he or she is 18 years old.
The additional impact of the Western diet

- Average Western diet:
  - rich in refined foods,
  - low in fermented foods, complex carbohydrates, fibre
- Refined foods are easily broken down in the upper GI tract
  - Thus very little left-over food makes it to the colon
- Colon is the site of most beneficial gut microbial activity
  - Starvation of this community can lead to ecosystem damage
  - ‘extinction events’ and reduced diversity
Save our Rainforests

Save our gut microbiota
Examples of diseases associated with reduced gut microbiota diversity (published research)

- Infant colic
- Autism
- Allergic asthma
- Eczema
- Inflammatory bowel diseases
- Celiac disease
- Colorectal cancer
- Obesity
- Neonatal necrotizing enterocolitis
- Irritable Bowel Syndrome
- Clostridium difficile infection
• Lack of microbial diversity
• Loss of ‘keystone’ species
• Overgrowth of opportunistic pathogens
• Poor diet/lifestyle
• Drug interactions

Looking inside the black box is the key to understanding disease

“Dysbiosis”
The human gut microbiota is a complex microbial ecosystem. Its function and behaviour is best studied as a whole.
The human colon is a type of chemostat…

…thus, chemostats can be used to emulate the human colonic environment.
• Seeded with fresh feces or defined communities and set to model the ecosystem of the colon
• Host-free system
• Can be used to ‘culture the unculturable’
• Can support whole gut microbial ecosystems for several weeks at a time

• We can model the gut microbiota under different stress conditions
• We can try to protect against the effects of stress
The good, the bad and the ugly

• **The Good**
  • Lactic Acid Bacteria (LAB)
    – E.g. *Bifidobacterium* and *Lactobacillus* spp.
  • Butyrate-producing bacteria
    – E.g. *Faecalibacterium prausnitzii*, *Roseburia* spp.

• **The Bad**
  • Opportunistic pathogens
    – E.g. *E.coli*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Bacteroides fragilis*
  • Sulfate-reducing bacteria
    – E.g. *Desulfovibrio* spp.

**The Ugly**: it really is not that clear-cut!
Some microbes are like bad teenagers in a subway station…

When the crowds are gone, they tend to start behaving in antisocial ways

E.g. *C. difficile*
**C. difficile** infection: a man-made disease

- Normal colon: *C. difficile* absent or numbers low
- Reduction in major genera of anaerobes: *C. difficile* grows to high numbers
- Antibiotics: clindamycin, cephalosporins, ampicillin
- Ulceration of colon
- Death
- Symptoms abate
- Return to normal
- Cessation of therapy

*Vancomycin or metronidazole*

Production of exotoxins A and B

Diarrhea

Ulceration of colon

C. Carlucci, A-V lab, 2012

Wikimedia commons
Our approach to fixing a damaged gut ecosystem...

- Isolation of a 33-strain, 25 species microbial *ecosystem* derived from a single, very healthy donor
  - Test for ecosystem stability *in vitro* (Robogut)
- Similar to the fecal transplant approach to treatment of gut disease
  - But is safer, more acceptable, more stable and completely defined

“RePOOPulate”
Microbes work better in teams

- Probiotic strains vs. probiotic ecosystems

Probiotic strains – single or few species acting alone

Microbial synergy: bugs support each other to create an overall larger benefit
“RePOOPulate”

- Acidaminococcus intestinalis
- Bacteroides ovatus
- Bifidobacterium adolescentis (x2)
- Bifidobacterium longum (x2)
- Collinsella aerofaciens
- Dorea longicatena (x2)
- Escherichia coli
- Eubacterium eligens
- Eubacterium limosum
- Eubacterium rectale (x4)
- Eubacterium ventriosum
- Faecalibacterium prausnitzii
- Lactobacillus casei
- Lactobacillus paracasei
- Parabacteroides distasonis
- Raoultella sp.
- Roseburia faecalis
- Roseburia intestinalis
- Ruminococcus torques (x2)
- Streptococcus mitis
- Likely novel species (x5)
- Likely novel genus & species (x1)
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Not just the usual probiotic subjects!
RePOOPulate proof-of-principle trial

- 2 elderly ladies with severe, recurrent *C. diff* infections were treated (April and June 2011)
- RePOOPulate made fresh at Guelph, driven to KGH, and administered via colonoscopy
  - 1 dose, 100mLs
- Both patients recovered within 2 days and have remained *C. diff*-free ever since (despite numerous subsequent antibiotic exposures)
In both patients, RePOOPulate signatures could be seen 6 months following administration: Perhaps colonization had taken place.
RePOOPulate

• A prototype for a new class of drug
  – Currently in development for a clinical trial (oral delivery)
• We are also developing a series of ‘Microbial Ecosystem Therapeutics’ products to treat different diseases
  – Different healthy host sources
  – Different host lifestyles for different donors?
How do we match patient to ecosystem?

Consider different people’s bodies as different models of car

Consider the gut microbiota as an engine
Maybe we should just replace the faulty part of the engine
Making better METs...

$^1$H NMR profiling of liquid gold

Goal: match the MET output to that of the native healthy community from which it is derived

Yen et al., Journal of Proteome Research, 2015
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**U Waterloo**
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Sandi Yen
I found the problem, Mr. Smith. Instead of probiotics, you have been taking amateur biotics.