What is a probiotic?

Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.

-World Health Organization, 2002
**Synbiotic**

Products containing both pre- and probiotics

**Prebiotic**

Non-digestible products that promote the growth of “healthy” gut bacteria

**Probiotic**

Live microorganisms administered in adequate amounts, confer a health benefit
A brief history of probiotics
The Bible states, “Abraham owed his longevity to the consumption of sour milk.”

In 76 BC, the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis.

1907 Metchnikoff proposed that the acid-producing bacteria in fermented milk, if consumed regularly, lead to a longer, healthier life.

Early 1930’s, in Japan, Minoru Shirota developed a fermented milk product called Yakult.

1965, New York, Lilly & Stillwell coined the term probiotic.
Where do probiotics come from?

Naturally occurring in some foods
Where do probiotics come from?

- Probiotics are actually members of a group of bacteria that reside within our intestines.
- This group of bacteria is called the Microbiota.
Microbiota
The actual “bugs” that reside within and on us.
Referred mostly to bacteria

Microbiome
Every bug, all of their genes, and everything they produce

Fungus – Mycobiome
Virus – Virome
Our microbial self

10^{14} or 100 trillion bacteria within our gastrointestinal tract

1:1 ratio with our own human cells

100-300:1 number of bacterial genes to our own human genes.
Our bacterial residents

**Stomach** $10^2$
- Lactobacillus
- Candida
- Streptococcus
- Helicobacter pylori
- Peptostreptococcus

**Duodenum** $10^2$
- Streptococcus
- Lactobacillus

**Jejunum** $10^2$
- Streptococcus
- Lactobacillus

**Large intestine (colon)**
- Proximal ileum $10^2$
  - Streptococcus
  - Lactobacillus

**Distal ileum** $10^8$
- Clostridium
- Streptococcus
- Bacteroides
- Actinomycinae
- Corneybacteria

**Colon** $10^{12}$
- Bacteroides
- Clostridium
- Bifidobacterium
- Enterobacteriaceae

Anatomy of Small Intestine
Your intestines have good & bad bacteria

**Bifidobacteria**
The various strains help to regulate levels of other bacteria in the gut, modulate immune responses to invading pathogens, prevent tumour formation and produce vitamins.

**Escherichia coli**
Several types inhabit the human gut. They are involved in the production of vitamin K2 (essential for blood clotting) and help to keep bad bacteria in check. But some strains can lead to illness.

**Lactobacillus**
Beneficial varieties produce vitamins and nutrients, boost immunity and protect against carcinogens.

**Campylobacter**
C. jejuni and C. coli are the strains most commonly associated with human disease. Infection usually occurs through the ingestion of contaminated food.

**Enterococcus faecalis**
A common cause of post-surgical infections.

**Clostridium difficile**
Most harmfully following a course of antibiotics when it is able to proliferate.
What does all of this bacteria do?

- **Boosts Immunity**
  - 90% of our immune system is located within the gut
  - Produces factors that replace mucus and prevent infections
  - Maintains an acidic pH

- **Supports healthy weight**
  - Produces chemicals that promote satiety

- **Improves mental health**
  - Synthesizes neurotransmitters

- **Increases energy levels**
  - Absorption of nutrients

- **Promotes cardiovascular health**
  - Improves cholesterol levels

- **Regulates hormones**
  - Estrogen, B12, folic acid and vitamin D
Diversity of the microbiome is acquired throughout youth

Your microbiome is given to you at birth. Our microbiome is imprinted during birth. The route of birth—whether bottle or breast fed—can influence the microbiome. As an infant develops, they will receive probiotics from the food that they ingest. Lactobacillus and other beneficial bacteria can help establish a healthy gut ecosystem.
we shape its composition...

DYSBIOSIS: Shifts in the composition, location or the function of your Microbiome
What is DYSBIOSIS?

"healthy" microbiome

composition
- 16S rRNA sequencing

function (purpose)
- Metagenomics
- Meta-transcriptomics
- Metabolomics

location
- Must sample different sites
The microbiome is stable AND resilient

Must sample different sites
Probiotic commensals

Bifidobacteria
Lactobacillus
Saccaromyces boulardi

Commensals known to overpopulate

Streptococcus
Clostridium
Klebsiella
Varicella
Spirochaetaceae

DYSBIOSIS
SCI and the gut

- UMN lesions
  - Decreased motility left colon
  - Spastic paralysis
  - Constipation, DWE, Incontinence
  - Fecal impaction proximal colon

- LMN lesions
  - Decreased motility left colon
  - Flaccid paralysis
  - Constipation, DWE, Incontinence
SCI and the microbiome

Mouse model of SCI  (Kigerl et al. Journal of Experimental Medicine Nov 2016, 213 (12) 2603-2620)
• Gut composition pre-injury affects outcome of SCI
• SCI caused an increase in gut permeability, increased inflammation and altered microbiota composition
• Feeding mice VSL3 probiotic reduced gut permeability, prevented inflammation and increased locomotor activity

• Found a reduction in butyrate promoting bacteria (Roseburia, Pseudobutyrivibrio, Dialister, Marvinbryantia and Megamonas) in UMN and LMN patients
SCI and the microbiome

MICROBIOME MAY EXACERBATE SCI

No treatment  →  Injury  →  Antibiotics to alter microbiome

Locomotor recovery was more significantly impaired
Exacerbated lesion pathology
Increased intraspinal inflammation

SCI and the microbiome

SCI ALTERS THE MICROBIOME

No treatment → Injury → altered microbiota composition
increase in gut permeability, increased inflammation

SCI and the microbiome

SCI ALTERS THE MICROBIOME

No treatment

Injury

Treat with probiotics

Prevented microbiota composition
Reduced gut permeability,
Decreased inflammation

SCI and the microbiome

Butyrate
Gut hormones
Brain

Mucus

Pre-injury

Post-injury

Loss of butyrate producing microbes

Bloodstream

Should you be taking a probiotic?

More than 40 diseases have been linked to bacterial imbalance including depression, arthritis, IBS, & cancer.
What do probiotics do?

**Protection**
- Disease-causing bacteria, viruses
- Toxins
- Cancer

**Absorption**
- Vitamins (B122)
- Minerals & Magnesium
- Glucose
- Fatty acids

**Production**
- Short chain fatty acids
- Vitamin K12
- B vitamins
- Enzymes

**Modulation**
- Antibody & immune cells
- Inflammation

**Function**
- Motility
- Bile & gastric secretions
- Immune function
Diseases proven to benefit from probiotics

- Infectious diarrhea
- Irritable bowel syndrome
- Antibiotic-associated diarrhea
- Weak immune system
- Eczema
- Ulcerative colitis
- Cholesterol
- Anxiety & depression
How probiotics work: preventing infection
How probiotics work: strengthening our barrier
How probiotics work: strengthening our barrier

Pre-carcinogens → Lactobacillus → Carcinogens

- Pre-carcinogens
- Enzymes in our bodies
- Carcinogens

Lactobacillus
Are probiotics safe?

Don't start taking probiotics without talking to your doctor especially if you have an immune deficiency or are being treated for cancer.

FDA does not monitor probiotics so a lot of variation and mis-marketing
What to look for...

Genus, species and strain of the microorganisms

- *Lactobacillus*
- *reuteri*
- ATCC55730
What to look for...

- **Number of organisms** contained in a single dose.

  Remember more is not always better

- **How often** you should take it and when (should be taken shortly after eating)

*Pick one that has at least 7 strains, & five billion CFU (colony forming units)*
What to look for…

How should this specific probiotic be stored

• refrigeration
• dark cool space

*always keep away from moisture and heat
What to look for…

“Viable through end of shelf life” vs “Viable at time of manufacture”

Encapsulated pills or other delayed-rupture technology ensure the bacteria survive the acidity of stomach & reach your colon.
What to look for…

Certification by an independent third party. The Food and Drug Administration (FDA) does not regulate most probiotics & therefore the amount of bacteria stated on the label might not be what’s actually in there.
Not all probiotics are created equal

**Bifidobacterium Bifidum**
- Supports production of vitamins
- Boosts immunity
- Prevents pathogens

**Lactobacillus acidophilus**
- Relieves gas, bloating
- Improves lactose intolerance
- Lowers cholesterol
- Reduces E. coli
- Vitamin K

**Bifidobacterium longum**
- Supports liver function
- Reduces inflammation
- Removes toxins

**Lactobacillus rhamnosus**
- Supports healthy skin
- Fights UTIs
- Reduces stress hormones and GABA neurotransmitter which reduces anxiety

**Saccharomyces boulardii**
- Yeast probiotic
- Effective at treating Crohn’s Disease
- Anti-toxin
- Anti-microbial
Probiotics and gut health is a major market

- Probiotics can be helpful in some cases but there are relatively few studies to tell us if and when they are effective in adults.
Problems with many probiotics

Not potent: 50% of all products do not contain the type or numbers of bacteria they claim

Not effective: Many probiotic supplements do not ensure that they get through the stomach acids and survive

Not natural: Many products are processed and have too much added sugar.
Pasteurization kills probiotics

Yogurt: best when “made with live, active cultures"

Avoid “heat treated after culturing”
Alternatives to probiotic supplements: Pre- and Syn-biotics

**PROBIOTICS**

**PREBIOTICS**

**SynBIOTICS**
Alternatives to probiotic supplements: fermented food

- Natto
- Kefir
- Kombucha
- Saurkraut
- Pickles
- Tempeh
- Lassi
Alternatives to probiotic supplements:

Fecal Microbiota Transplants
Fecal Microbiota transplants

• Have been used to treat C. difficile infections
  o >90% efficacy compared to antibiotics (~50%)

• Currently a number of clinical trials evaluating FMT in Inflammatory Bowel Disease
  • Results have been mixed so far
Still many unknowns for FMT

Future of probiotic supplements

Lacto-ceuticals using the fermentation process with different types of food, such as whey

Genetically modified Lactobacillus
Interrogating the microbiome & immunity in recurrence of ileal disease post resection
Scott Lee, MD, UWGI
William DePaolo, PhD, UW CMST

This study aims to further evaluate and define immunological, metabolic or inflammatory signatures that predispose patients with Crohn's disease to post-surgical disease recurrence as compared to those patients who do not have significant post-surgical recurrence.

Our goal is to create a hypothesis of how metabolomics influence and can predict recurrence of Crohn’s post-surgically. This will lead to more focused and refined studies to better define this question.

Dr. Scott Lee is an associate professor of medicine with expertise in inflammatory bowel disease (Crohn’s disease and ulcerative colitis). His research is focused on inflammatory bowel diseases (IBD) including – new therapies for IBD, improving outcomes in the treatment and long term management of IBD, evaluation of non-invasive biomarkers to assess disease activity in IBD patients and the effects of the microbiome on IBD.

HIV-exposed microbiome impacts the severity of co-infection
Patricia Pavlinac, PhD, UW Global WACH
William DePaolo, PhD, UW CMST

Patricia Pavlinac, PhD MS, is an epidemiologist and co-director of the Healthy Growth & Development Core of the Global Center for Integrated Health of Women, Adolescents, & Children (Global WACH). Dr. Pavlinac’s research aims to identify interventions to halt morbidity and mortality attributed to enteric and diarrheal diseases. Her other research interests include pediatric tuberculosis, particularly the diagnosis of tuberculosis in pediatric populations.

DIET & NUTRITION

Members of DePaolo Lab
For information about CMiST’s programs and how to support our research and art initiatives please visit

https://cmistuw.org/ways-to-help/
The Microbiome and SCI

Rina Reyes, MD
SCI Physician, VA Puget Sound Health Care System
Associate Professor, Rehabilitation Medicine
University of Washington
Relevance to SCI

• Multiple SCI health conditions
  – Many potential target conditions for microbiome interventions

• Frequency of antibiotic treatment:
  – Is gut dysbiosis inevitable after SCI?
  – Rise of antibiotic resistance
  – Risk for *C. difficile* (“C. diff”) diarrhea

What is the role of probiotics and gut dysbiosis in SCI health?
Probiotics and SCI: What we do (and don’t) know

• Limited but growing body of research evaluating microbiota/microbiome and role of probiotics in SCI

• Three areas of noteworthy research
  – Neurogenic bladder
  – Neurogenic bowel
  – Neuroprotection/recovery
Probiotics and SCI: Neurogenic Bladder

- Defining the urine microbiome with and without SCI
  - 2 papers (Groah, Fouts and colleagues 2012, 2016)
    - Same population, examined with different analytical resources in a cross-sectional study
    - 47 subjects (24 with neuropathic bladder, 23 controls)
      - All without symptoms of UTI
    - DNA genus vs. species level analysis
    - Urinalysis, urine culture

- Urine microbiome showed differences according to gender and bladder function
Neurogenic and Non-neurogenic Bladder: Defining the Microbiome (Groah et. al)

Finding # 1

• **ALL** samples had bacteriuria by DNA PathoScope analysis
  • Only 23 had positive urine culture
  • E. coli was most commonly found

• Non-neurogenic bladder
  • Women: higher proportion of *Lactobacillus crispatus*
  • Men: higher proportion of *Staphylococcus haemolyticus*, streptococcal organisms

• What does this mean?
  • Healthy urine is not sterile!
Neurogenic and Non-neurogenic Bladder: Defining the Microbiome (Groah et. Al)

Finding #2: Women with NGB

- Different lactobacillus community than women without NGB
  - Absent *L. crispatus*
- Higher proportion of:
  - *Lactobacillus* (and *L. iners*)
  - *Gardnerella* (and *G. vaginalis*)
  - *Enterobacter*
Neurogenic and Non-neurogenic Bladder: Defining the Microbiome (Groah et. Al)

Finding #3: Men and women with NGB

- Greater proportion of certain bacteria
  - *Enterococcus faecalis*
  - *Klebsiella pneumonia*
  - *Pseudomonas aeruginosa*
  - In addition to *E. coli*
- Subjects using CIC or SPC had higher *Enterobacter* proportion than subjects who voided
- 4 subjects had *Actinobaculum sp.* only by DNA PathoScope and not in culture
- ALL associated with high WBC in urine
SCI Urinary Microbiome Changes Over Time

• Bossa et. al 2017
  – Followed 3 subjects with SCI, chronic catheterization over time before and after probiotics treatment
  – Findings from catheter biofilm samples
    • Unique microbiome
    • Composition changed before clinical UTI diagnosis
    • Probiotics changed community transiently; native community was resilient

• Nally et. al 2018
  – *Burkholderia fungorum* in individual with augmented bladder during healthy and disease states found only by DNA sequencing
Urine microbiome: Implications

• Healthy urine has a bacterial community
  – Neurogenic bladder leads to a different microbial community in host with SCI
  – Redefines UTI and goal of treatment
    • Asymptomatic bacteria in urine
    • Goal is not necessarily sterile urine

• High urine WBC may not indicate a disease state
  – Possibly decrease significance or disregard when diagnosing catheter-associated UTI (“CAUTI”)

• What about targeted microbiome regulation or manipulation?
Neurogenic Bladder: Bacterial Interference

• “Use of bacteria of low virulence to compete with and protect against colonization and infection by disease-causing organisms.” (Darouiche 2012)

• Passive interference: non-treatment of resident bacteria when host shows no symptoms prevents UTI

• Active interference: deliberate introduction of “benign” bacteria to prevent colonization by disease-causing bacteria
Bacterial Interference and Probiotics: Possible Mechanisms

- Competition for nutrients
- Competition for binding sites
- Antibacterial substance production
- Immune modulation
- Genetic expression regulation
- Biofilm disruption
Bacterial Interference, NGB & UTI

• Earlier studies promising → multicenter, randomized, controlled trial 2011
  – Used more “benign” *E. coli* strains to establish colonization of urine by introducing into bladder

• Evaluated rate of UTI
  – ‘Evaluable’ if remained colonized > 4 weeks, followed monthly x 12 months
Study Flow

59 subjects

49- E. coli

Colonized?

Study

 Repeat x 3 if not

Colonized

Study

Not colonized

Not reported

15- saline

Not colonized?

Study

Only 27 evaluable (17 experimental, 10 control)
Findings:

- **Limited colonization success:**
  - 38% colonization rates
  - None of 5 female subjects
  - Earlier studies had better success rate

- **Decrease in UTIs:**
  - 5 of 17 (29%) experimental subjects vs. 7 (70%) of controls had at least 1 UTI in f/u year
  - Average # UTIs per patient year lower in experimental (0.5) vs. control (1.68) group
  - Drop in UTI rates mirrored earlier findings
Bacterial Interference, NGB & UTI  
(Darouiche et. al 2011)

Findings, continued

• Reasonably good protocol safety
  • No UTIs attributed to E. coli strain used for inoculation
  • Earlier studies: no sepsis, 1 AD event, 1 unrelated UTI

• Poor acceptance, adherence to inoculation protocol
  • Large drop-out rate
  • Limits practical application
  • Reduces quality of data, analysis
Bacterial Interference, NGB, UTI (Sunden et. al 2010)

- Randomized, blinded, controlled, crossover design
- Inoculation of E. coli vs. saline into bladder
  - Re-inoculation required in a few
- 20 subjects completed study

Findings: suggest efficacy, safety

- Time to first UTI longer (11.3 vs. 5.7 months) during treatment vs. saline
- Fewer UTIs reported by treatment group (13) vs. saline (35)
- No pyelonephritis (kidney infection)
Local Bacterial Interference, NGB, UTI
(Trautner 2003, 2007; Prasad 2009)

- *E. coli* 83972 prevented catheter colonization by an array of pathogenic organisms, so its biofilm may be protective.

- Foley catheters immersed in suspension with *E. coli* before insertion
  - Unsuccessful colonization when *Proteus* present
  - No UTIs attributed to this *E. coli* strain

- Viable for subjects who use intermittent catheterization
  - 3 days of indwelling catheter for colonization
  - 8 of 14 subjects (62%) successfully colonized > 3 days after removal
  - UTI rate dropped from 2.27 per patient year to 0.77 after intervention
But wait!
How Strong is the Evidence?

• Cochrane review (Toh SL et. al 2017)
  – included only 3 studies based on design (Darouiche 2005 and 2011; Sunden 2010)

• Concluded high risk of bias in reported results, with effectively very low evidence quality

• Therefore, uncertain if probiotic instillation into the bladder prevents UTI in people with SCI.
Considerations and Future Directions: Bacterial Interference for NGB & UTI

• Intriguing results, important steps in exploring interference as a solution
• Highlights challenges in this area of research, lack of studies rated as high quality of evidence
• Need to address practicality of protocol
• Investigate
  – Other methods of delivery
  – Molecular basis for bacterial interference
  – Differential effect of E. coli interference on women with SCI
What about the Microbiome and Probiotics for Neurogenic Bowel?

- Defining the Intestinal Microbiota after SCI (Gungor 2016)

- Non-neurogenic bowel: dominant communities ferment non-digestible carbohydrates to short chain fatty acids like butyrate
  - epithelial cell growth/development, immune function, anti-inflammatory effects on macrophages, suppress ongoing inflammation in central nervous system

- Evaluated DNA from stool samples
  - 30 subjects with SCI (15 UMN, 15 LMN)
  - 10 controls

- Butyrate-producing bacterial levels are reduced in neurogenic bowel
Bacterial Interference & the Gut in SCI

• Antibiotic-associated and *C. difficile* diarrhea
  – *Lactobacillus casei* Shirota probiotic (Wong 2014)

• 164 subjects needing antibiotics randomized to receive or not receive probiotics

• Association found between diarrhea and:
  – no probiotic treatment
  – poor appetite

• More rigorous study needed

• Systematic review underway
Bacterial Interference and the Gut after SCI: *C. diff* diarrhea

- Fecal transplantation and SCI
  
  (Brechmann et. al 2015)
  
  - One published case report of man with incomplete tetraplegia and recurrent *C. diff* infection
  - Colonoscopic stool transplantation
  - Developed sepsis-like syndrome requiring multi-drug antibiotic treatment
  - Despite this, no relapse at 12 week f/u
Microbiota and SCI Neuroprotection

• Kigerl, Popovich et. Al (201, 2018)

• Gut microbiota interact with nervous system in healthy state
  – Via immune cells (Gut-associated Lymphoid Tissues or “GALT”)
  – By secreting neuroactive metabolites that affect brain, spinal cord function (butyrate, choline, GABA, serotonin, dopamine, acetylcholine)

• Bacterial translocation or “leaky gut” with dysbiosis
Microbiota and SCI Neuroprotection

- SCI-related gut dysbiosis in mouse model associated with:
  - Bacterial migration across gut wall
  - Activation of GALT immune cell function, more inflammatory markers
  - Worsening of intraspinal inflammation
  - Change in composition of gut microbiome
  - Impaired functional recovery (locomotor scores)
Microbiota and SCI Neuroprotection

Probiotic feeding in mouse model had protective effect and improved recovery from SCI.

• Gut dysbiosis induced after antibiotic treatment, followed by SCI
• Motor recovery, spinal cord samples compared to rats without gut dysbiosis who had experimental SCI
  – Less locomotor recovery, white matter tracts spared in mice with antibiotic dysbiosis before SCI.
  – No difference in locomotion if dysbiosis induced 2 weeks after SCI.
Probiotics and SCI Neurorecovery (Kigerl et. al)

- Medical-grade probiotics given after SCI in mice
  - started immediately after SCI and daily x 35 days
- Versus controls, treated mice had:
  - Better locomotor recovery
  - Reduced lesion volume
- But effects ? time-sensitive
- Experiment repeated with new batch of probiotics, mice, separated by 1 year with similar benefit
Microbiome, Probiotics and SCI Neuroprotection

• Data very preliminary

• Manipulation of gut’s microbiome via probiotics may have therapeutic value after SCI in mice, although mechanism unclear
Burning Questions

• Is gut dysbiosis another medical complication after SCI?

• Are gut, bladder microbiomes and dysbiosis suitable new targets for treatment to improve SCI health, function?

• Will advancements in characterization and detection of microbiome changes serve as markers of health and disease, inform clinical decisions?
Implications for SCI

• Data is intriguing, but good quality data is limited and preliminary
• SCI conditions stand to potentially benefit from advancement in knowledge about probiotic effects on human health
• Many challenges and opportunities to studying probiotics
  – designing practical applications and high quality investigations
• Translation of bench and animal model research to humans critical
Recommendations

• Defend and feed your microbiome!
  – Judicious antibiotic use
  – Appropriate interpretation of culture results
  • Remember: healthy urine is not sterile
  – Prebiotics, fiber, natural probiotic sources
  – Focus on nutrition during antibiotic treatment
  – Consider probiotics; speak with your medical provider

• Manage expectations about health benefits of probiotics
• Be curious, stay informed about progress in these areas
Questions?