Fresh infusions: The science behind fecal transplants

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Texas Children’s Hospital
A brief history of fecal transplantation

• **4th century, China**
  – Ge Hong described administration of a human fecal suspension by mouth for food poisoning or severe diarrhea

• **16th century, China**
  – Li Shinzen described “yellow soup”, a solution of fermented, dry, or infant feces use to treat fever, severe diarrhea, pain, vomiting and constipation

• **17th century, Italy**
  – Fabricus Aquapendente described the use of fecal therapy in veterinary medicine

• **20th century, USA**
  – Eisenman et al. 1958, Surgery: Fecal enema therapy in 4 patients with pseudomembranous colitis – prompt recovery, discharged within days
What we currently know about FMT

- **It can be highly effective for CDI**
  - Success rates >90% for patients who have failed successive rounds of antibiotic therapy
  - Resolution in 89% of cases following a single treatment
  - Adverse effects/events are uncommon

- **FMT protocols vary widely**
  - Means of administration
  - Quantity of material used
  - Duration of treatment

- **Relatives appear to be slightly better donors**
  - Resolution with relative-donors: 93%
  - Resolution with non-relative donors: 84%

- **Currently FDA requires IND application for FMT**
  - FMT for CDI permitted
  - IND required for all other potential FMT applications

References:

Gough et al. 2011 Clin Infect Dis
Smith et al. 2014 Nature
FMT at Texas Children’s Hospital

• Donor screening began in October/November 2012
• First transplants conducted in November 2012
• Protocols for *Clostridium difficile* infection and Ulcerative colitis
• IND for Ulcerative colitis in final stages of approval
Healthy donor screening process

- **Stool testing:**
  - *Clostridium difficile* (by PCR)
  - EHEC (by PCR)
  - Routine bacterial culture for enteric pathogens (*Salmonella*, *Shigella*, *E. coli 0157:H7*, *Campylobacter*, *Yersinia*)
  - *EHCE, Shigatoxin, H. pylori, Giardia* and *Cryptosporidium* antigen
  - Ova and parasites

- **Serologic testing:**
  - HIV (types 1 and 2)
  - Hepatitis (A, B, C)
  - Rapid plasma reagin
  - Liver panel

- **Health questionnaire:**
  - Assesses clinical and demographic variables and exclusion criteria
  - Possible pathogen exposure opportunities
  - History of GI symptoms, other health issues and medications, etc.
Preparation of donor stool

- Filter
- Centrifuge

- Resuspended in glycerol
- Frozen at -80°C
- Stored up to 8 weeks

At time of transplant, sterile saline is added and preps are warmed to body temperature.
What is the 16S rRNA gene?

The gene encoding the small ribosomal subunit in Bacteria/Archaea

Why it is used in microbiome studies:

• **Highly conserved**: every bacterium/archaeon has at least one copy

• **Taxonomically informative**: conserved and highly variable regions allow for taxonomic identification at multiple taxonomic levels

Images from wikipedia.org and www.alimetrics.net

| CONSERVED REGIONS: unspecific applications |
| VARIABLE REGIONS: group or species-specific applications |
Evaluating donor stool

Donor specimens and preps cluster together relative to one another in PCOA space.

Donor specimens and preps share ~70-97% similarity with one another, depending on the metric used.
What does a healthy fecal microbiome look like?

16S profiles summarized at the phylum-level

Hollister et al., submitted
What does a healthy fecal microbiome look like?

Genus-level

16S rRNA gene libraries

Shotgun metagenomes

Hollister et al., submitted
Evaluating donor stool

Donor specimens bear strong resemblance to those characterized by the HMP.
What can a sick community look like?

Stool communities from cases of antibiotic-associated diarrhea

Hollister et al., unpublished
What can a sick community look like?

Stool communities from a variety of suspected CDI cases

- Nonrecurrent (n=24) → Cases with single episode of CDI, no relapse reported
- Recurrent (n=15) → Cases of recurrent CDI
- Healthy subjects (n=43) → Houston-based volunteers from the NIH Human Microbiome Project

Hollister et al., unpublished
What can a sick community look like?

Stool communities from a variety of suspected CDI cases

The family that contains *C. difficile*

- Nonrecurrent (n=24) → Cases with single episode of CDI, no relapse reported
- Recurrent (n=15) → Cases of recurrent CDI
- Healthy subjects (n=43) → Houston-based volunteers from the NIH Human Microbiome Project

Hollister et al., unpublished
C. difficile comprises a relatively small part of the expansion of Peptostreptococcaceae

Hollister et al., unpublished
What should our restoration end point(s) be?

- Resolution of symptoms
  - Return to healthy GI function
  - Discontinued need for antibiotic therapy

- Restoration of GI community
  - Structure
  - Function
  - What degree of restoration = success?
  - Should donor matching be considered?
**Ulcerative colitis protocol**
37 treatments over 12 months

- FMT via colonoscopy
- Day 2-14 (daily) FMT via retention enema
- Weeks 3-4 (3x per week) FMT via retention enema
- Weeks 5-12 (weekly) FMT via retention enema
- Week 14 colonoscopy
- Months 4-12 (monthly) FMT via retention enema

**C. difficile protocol**
1-2 treatments over 3-5 days

- FMT via colonoscopy
- No resolution of symptoms in 3-5 days
- Resolution of symptoms in 3-5 days
- Stool analysis post-FMT (2 weeks & 2 months)
## FMT at Texas Children’s Hospital

<table>
<thead>
<tr>
<th>Case</th>
<th>Demographics</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>14 yo male</td>
<td>Ulcerative colitis, surgery, recurrent <em>C. difficile</em> unresponsive to</td>
</tr>
<tr>
<td></td>
<td><em>C. difficile</em></td>
<td>treatment, symptoms resolved post-FMT.</td>
</tr>
<tr>
<td></td>
<td>infection</td>
<td></td>
</tr>
<tr>
<td>CD2</td>
<td>16 yo female</td>
<td>Wisdom teeth removal, antibiotics given for abscess formation, *C.</td>
</tr>
<tr>
<td></td>
<td><em>C. difficile</em></td>
<td><em>difficile</em> unresponsive to treatment, attempted FMT at home,</td>
</tr>
<tr>
<td></td>
<td>infection</td>
<td>symptoms resolved post-FMT at TCH.</td>
</tr>
<tr>
<td>UC1</td>
<td>16 yo male</td>
<td>Clinical remission at start of study, received all 28 treatments (12w)</td>
</tr>
<tr>
<td></td>
<td>Ulcerative colitis</td>
<td>and remained in remission for 16 weeks.</td>
</tr>
<tr>
<td>UC2</td>
<td>13 yo male</td>
<td>Clinical remission at start of study, difficulty with retention enemas,</td>
</tr>
<tr>
<td></td>
<td>Ulcerative colitis</td>
<td>patient removed from protocol during Week 7.</td>
</tr>
<tr>
<td>UC3</td>
<td>15 yo male</td>
<td><em>C. difficile</em> infection prior to start of study, CDI treated, completed</td>
</tr>
<tr>
<td></td>
<td>Ulcerative colitis</td>
<td>of 25 of 28 treatments, and achieved remission for 11 weeks.</td>
</tr>
<tr>
<td>UC5</td>
<td>14 yo female</td>
<td>Gradually weaned off steroids during first 2 weeks of treatment,</td>
</tr>
<tr>
<td></td>
<td>Ulcerative colitis</td>
<td>completed 23 of 28 treatments, and achieved remission for 11 weeks.</td>
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</table>
FMT for *Clostridium difficile* infection

Case studies from two pediatric CDI patients

![Graph showing changes in genus-level relative abundance pre-FMT, 2 weeks post-FMT, and 2 months post-FMT for Donor, Patient 1, and Patient 2.](image)
FMT for *Clostridium difficile* infection

Case studies from two pediatric CDI patients

- Immediate decline in reads associated with *Escherichia/Shigella*
- Immediate decline in reads associated with *Streptococcus*
- Gain of reads associated with *Lachnospiraceae, Clostridium, and Bacteroides*
FMT for *Clostridium difficile* infection

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- Immediate decline in reads associated with *Escherichia/Shigella*
- Immediate decline in reads associated with *Streptococcus*
- Gain of reads associated with *Lachnospiraceae, Clostridium, and Bacteroides*

Patient 1’s similarity to prep community increased from 25% pre-FMT to 40% at 2 months post-FMT *

Co-morbid ulcerative colitis

* Weighted Unifrac metric of 16S rRNA gene sequence libraries (V3V5)
FMT for *Clostridium difficile* infection

Case studies from two pediatric CDI patients

- Immediate decline in reads associated with *Escherichia/Shigella*
- Immediate decline in reads associated with *Streptococcus*
- Gain of reads associated with *Lachnospiraceae, Clostridium, and Bacteroides*

Patient 2’s similarity to prep community increased from 13% pre-FMT to 60% at 2 months post-FMT *

* Weighted Unifrac metric of 16S rRNA gene sequence libraries (V3V5)
Is 40-60% similarity a reasonable target?

- Healthy adult vs. Healthy adult = 40-50% similarity
- Healthy child vs. Healthy child = 40-50% similarity
- Healthy child vs. Healthy adult = ~40% similarity

Khoruts et al. 2010 J Clin Gastroenterology

Hollister et al., submitted
FMT for *Clostridium difficile* infection

Which bacteria flourish with FMT and/or originate from the donor?

Likely candidates:

<table>
<thead>
<tr>
<th>OTU</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTU-462</td>
<td>Veillonella dispar</td>
</tr>
<tr>
<td>OTU-1399</td>
<td>Bacteroides plebius</td>
</tr>
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<td>OTU-1772</td>
<td>Bacteroides ovatus</td>
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<td>OTU-638</td>
<td>Dialister invisus</td>
</tr>
<tr>
<td>OTU-1771</td>
<td>Bacteroides vulgatus</td>
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<tr>
<td>OTU-584</td>
<td>Faecalibacterium prausnitzii</td>
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<td>Eubacterium rectale</td>
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<td>OTU-1959</td>
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<tr>
<td>OTU-902</td>
<td>Clostridium sp</td>
</tr>
<tr>
<td>OTU-1129</td>
<td>Clostridium paraputricifum</td>
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Gained by CDI Subject 1

Gained by CDI Subject 2

Gained by both CDI recipients

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## FMT for *Clostridium difficile* infection

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### Table 1: Composition of stool substitute (RePOOPulate)

<table>
<thead>
<tr>
<th>OTU</th>
<th>Species</th>
<th>% identity to closest match</th>
<th>Relative abundance (by biomass) in RePOOPulate formulation</th>
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<tbody>
<tr>
<td>OTU-462</td>
<td>Veillonella dispar</td>
<td>98.93</td>
<td>++</td>
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Petrof et al. 2013 Microbiome
FMT for *Clostridium difficile* infection

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Khoruts et al. 2010 J Clin Gastroenterology; Petrof et al. 2013 Microbiome
Patient experienced complete remission for 16 weeks post-treatment.
Patient had difficulty with retention enema (30 of required 60 min).

Patient was removed from FMT protocol on day 46.
Patient remained in remission throughout therapy and for 11 weeks after discontinuation of treatment.

Treatments stopped early due to FDA ruling requiring IND application.
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OTU-1723: Eubacterium rectale
OTU-1902: Parabacteroides diastonis
OTU-638: Dialister invisus

FMT for Ulcerative colitis

Who flourish with FMT and/or originate from the donor?

Khoruts et al. 2010 J Clin Gastroenterology; Petrof et al. 2013 Microbiome
FMT for Ulcerative colitis

Evaluating success

- FMT may not work for in all cases
  - Compliance with protocol
  - Duration of treatment
  - Specific donor matching may need to be considered

- Resolution of symptoms
  - Most subjects remained off of all medication for 11 weeks (or more) following treatment
  - Shifts observed in microbial community, as well as in host gene expression
  - Effects were not permanent
  - May require longer duration of treatment or regular “boosters”
The developing science of FMT

- Our understanding of FMT continues to develop
  - Shifts in community composition are dramatic but not complete
  - Mechanism(s) underlying efficacy are uncertain
  - Information regarding long-term outcomes is limited but promising

- What we’ve learned so far
  - Patients want access to FMT
  - CDI is relatively “easy” to treat with FMT, while other conditions (e.g., UC) may require more intensive therapy
  - FMT triggers microbial community shifts in stool and along the mucosa; and it induces shifts in host gene expression

Hollister et al., Gastroenterology 2014 doi: 10.1053/j.gastro.2014.01.052
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