

**make
history.**



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Oral Microbiome Transplantation, the Future?

DHK. Ketagoda, S. Nath, L. Jamieson, L. Weyrich, Peter Zilm.

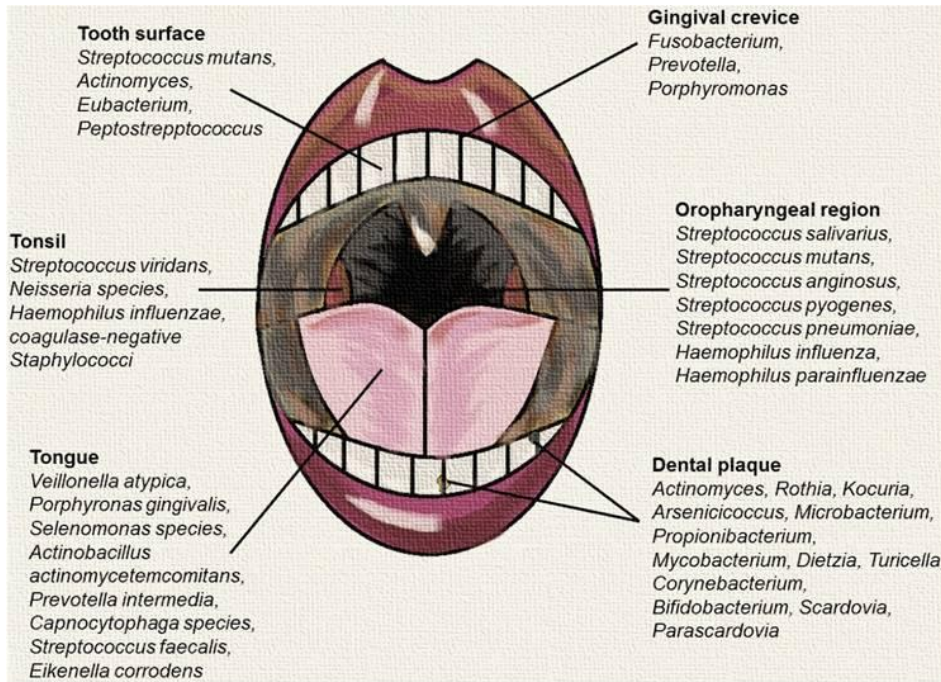
Australasian College for Infection Prevention & Control International Conference (ACIPC 2024)

**Assoc. Prof. Peter Zilm, Translational Research
in Oral Health Science.**

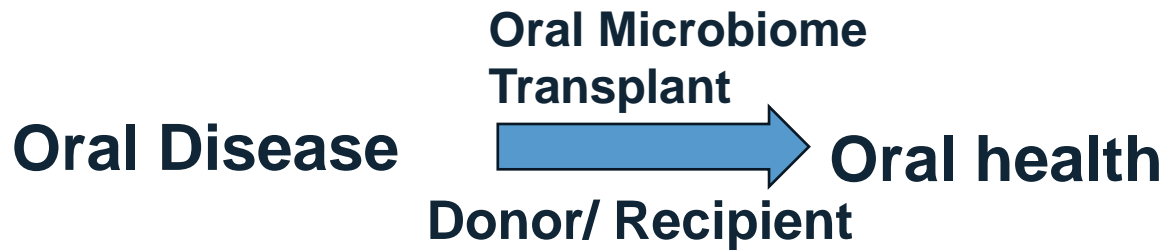
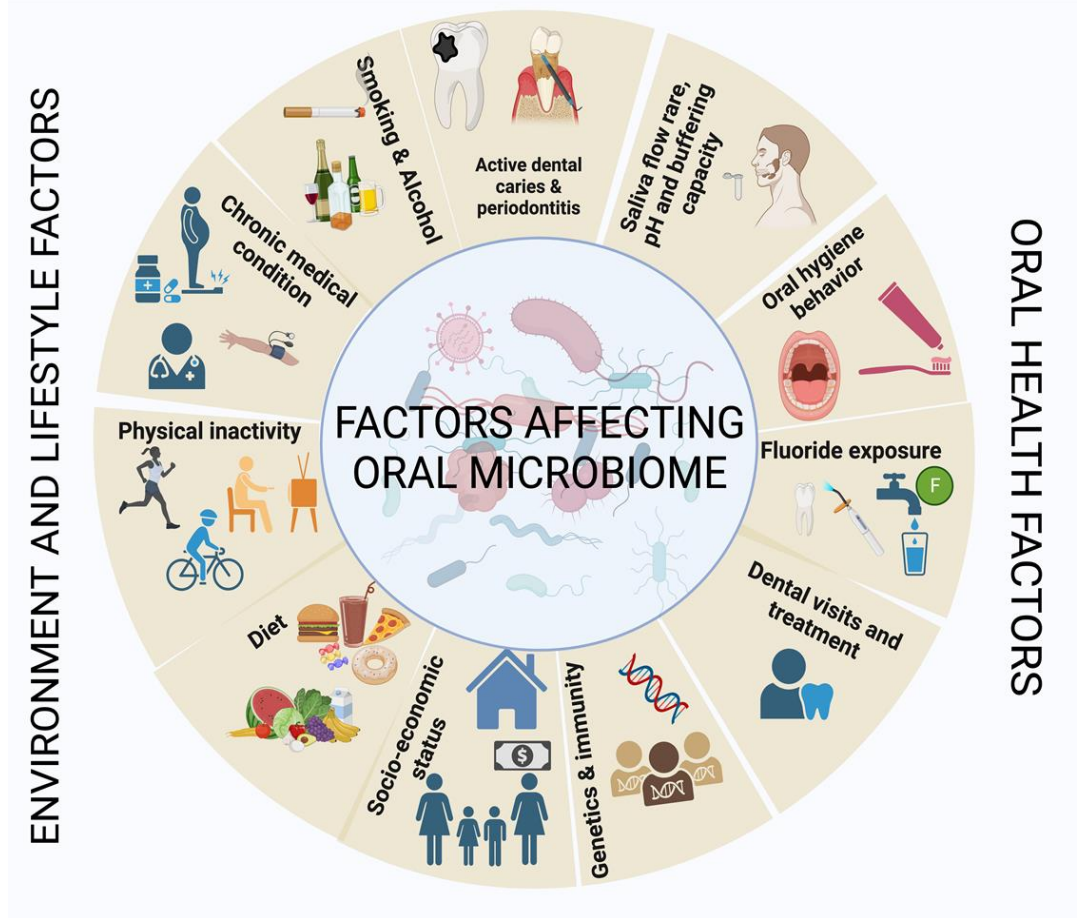
<https://health.Adelaide.edu.au/our-research/translational-research-in-oral-health-science>
Microversal LLC



- The oral environment maintains the second largest microbiome in the human body consisting of around 700 taxa including bacteria, fungi and viruses.



+



- **Oral Microbiota / Microbiome = Microbial communities that maintain homeostasis**
- **Changing environmental conditions will lead to the emergence of pathobionts**

Dental Caries

- Dental decay (caries) is one of the most prevalent health diseases, sixth most costly health condition globally
- Caused by the fermentation of sugar to lactic acid by oral bacteria
- Tooth enamel and dentine is hydroxyapatite, solubility of 30mg/L at neutral pH 7 to 30,000mg/L at pH 4.
- **Our current strategies simply do not prevent caries.**



William Addis 1780



OMT - From Bench to Chairside

Objective

- Can oral microbiome transplantation (OMT) be used clinically to prevent dental caries?
 - Who are OMT donors ?
(*Super Donor Assessment Tool*)
 - Can the oral microbiome be grown *in vitro*.
 - Time, **Species diversity**. Biobank storage
 - Does OMT Work?
 - **Animal model**, Frequency of OMT.
 - **Does OMT change the oral microbiome in the recipient?**
 - **Is OMT safe?** - CRP, gut histology/metabolome

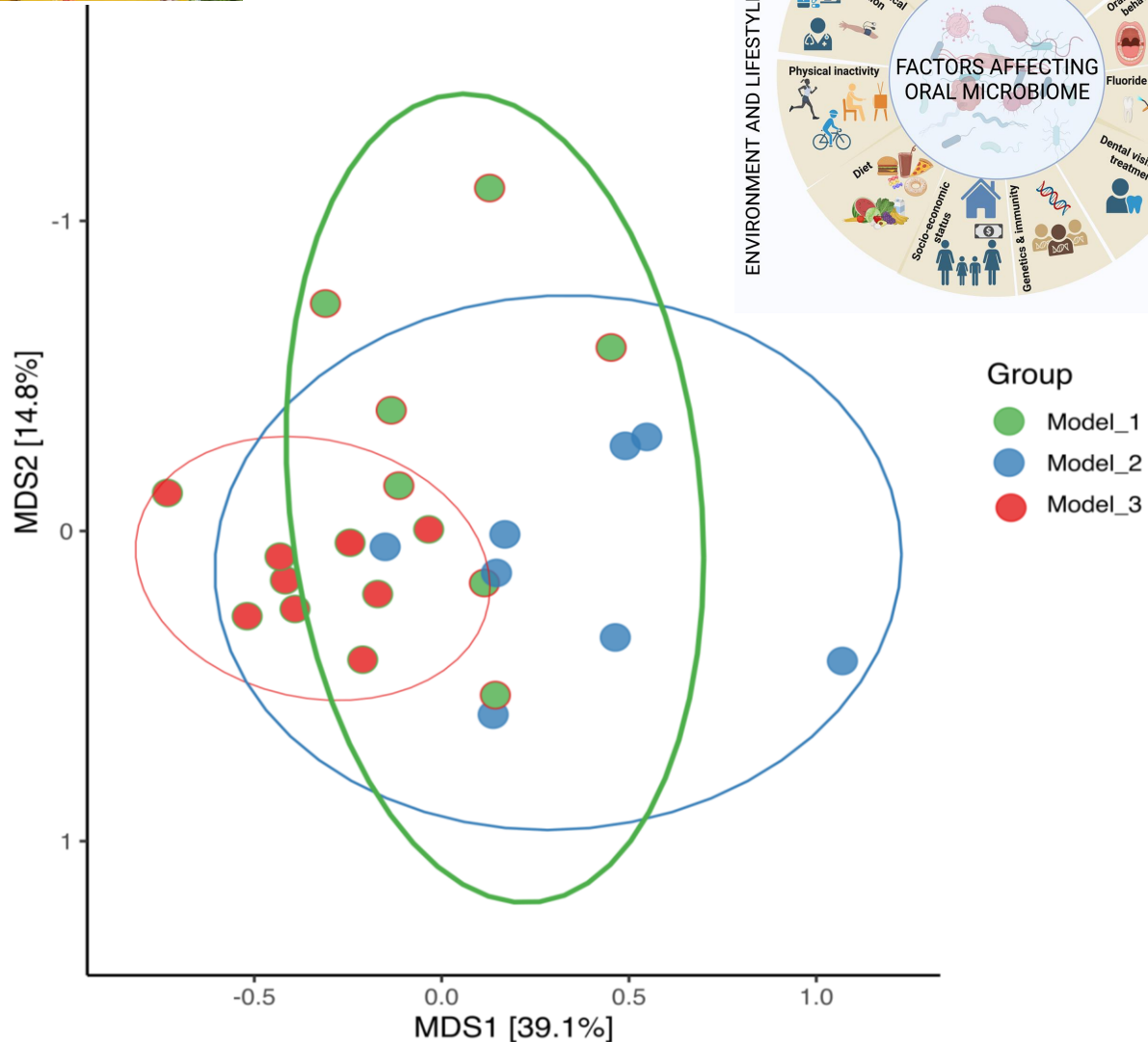
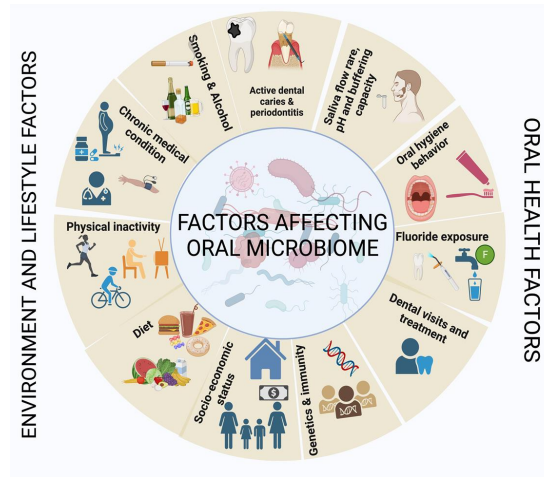
re•search,
[ri-surch, ree-surch], n

1. what you are doing
when you don't know
what you're doing.

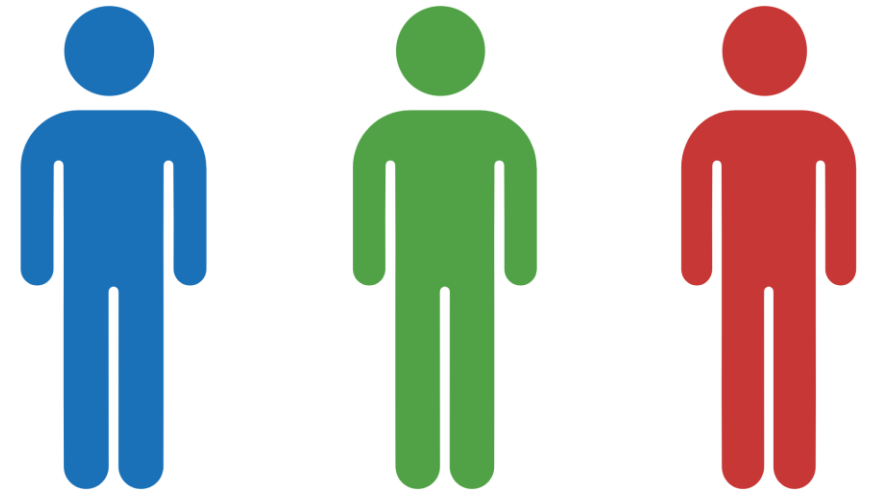




Who are OMT donors?



24 samples & 59 taxa (Genus). PCoA tax_transform=log2 dist=bray



Optimal donor
Poor oral & lifestyle habits but good dental health

Best donor
Good oral & lifestyle habits & good dental health

Suboptimal donor
Poor oral & lifestyle habits & history of past dental caries

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Characterising healthy Australian oral microbiomes for 'super donor' selection

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ARTICLE INFO

Keywords:
16S rRNA sequencing
Diet
Demographic
Oral microbiome
Oral health
Dental plaque
Public health

ABSTRACT

Objectives: Among healthy people, we understand very little about the sociodemographic, lifestyle, and dental hygiene behaviours that shape their oral microbiota. This study investigates how sociodemographic, lifestyle and dental hygiene behaviours shape oral microbiota diversity and composition in an Australian population to better inform healthy oral microbiota donors for Oral Microbiota Transplantation (OMT).

Methods: The study comprised 93 healthy adults who underwent comprehensive oral examinations and questionnaires to assess their health status. Participants were excluded if they had any active systemic or oral disease. All completed a questionnaire containing information on socio-economic, lifestyle, behavioural, and oral health factors. Supragingival plaque was collected, and 16S ribosomal RNA (rRNA) amplicon sequencing was used to analyse microbial composition. Associations between the oral microbiome alpha (within-sample) beta

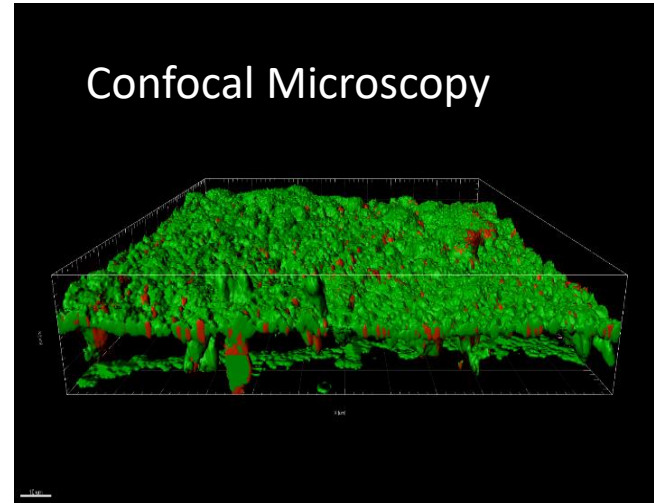


Can the oral microbiome be grown *in vitro*?

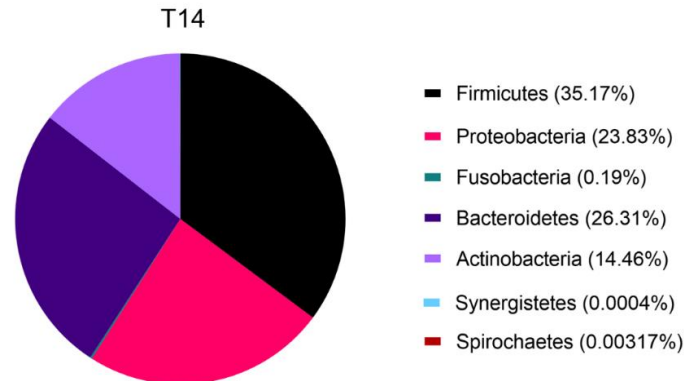
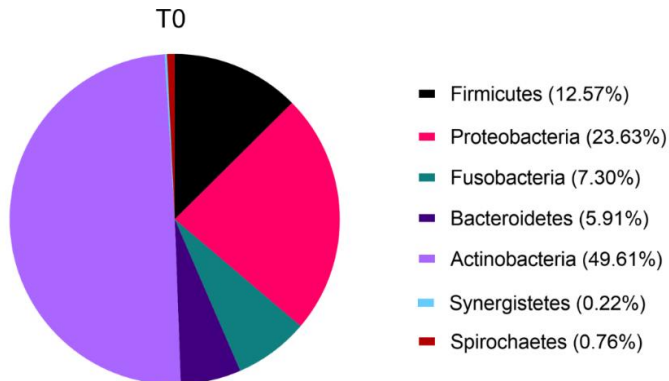
Approval from the University Adelaide Human Research Ethics Committee (H-202-34609)



- Human plaque grown on hydroxyapatite discs using Artificial saliva medium for 10 days
- Monitor biofilm growth and α - and β -diversity



Journal of Microbiological Methods 223 (2024) 106961



Development of an *in vitro* biofilm model of the human supra-gingival microbiome for Oral microbiome transplantation

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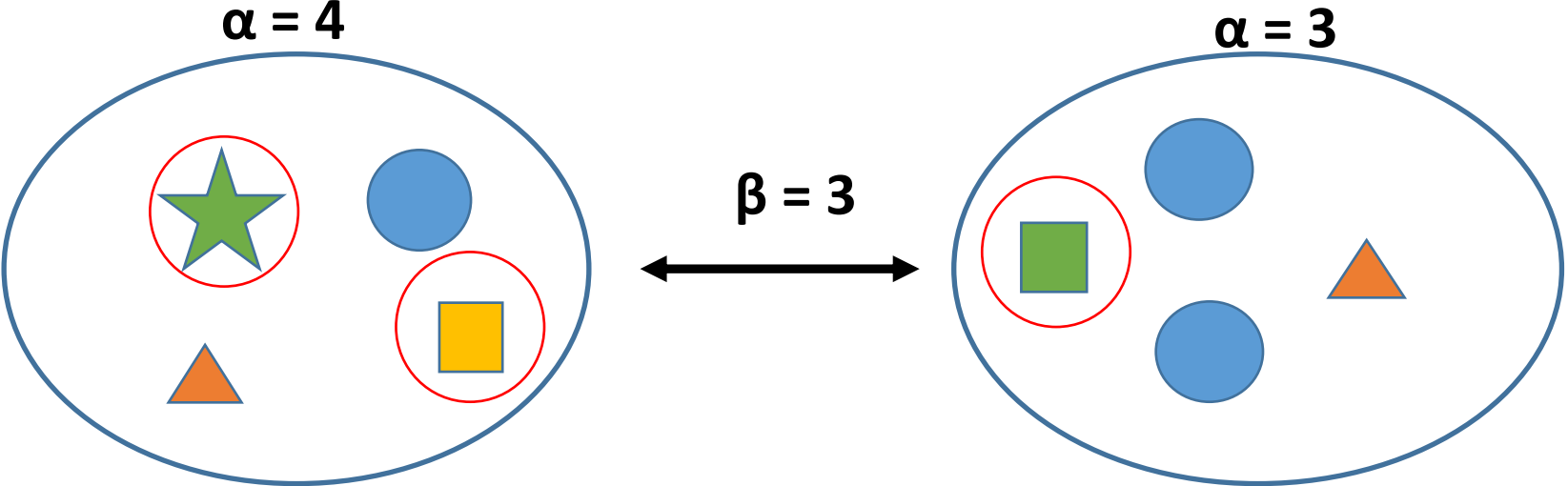
ARTICLE INFO

Keywords:
 Dental plaque
 DNA sequencing
 Oral microbiome transplantation
 Flow cell
 Microbiota
 Oral bacteria
 Species diversity

ABSTRACT

The high prevalence of dental caries and periodontal disease place a significant burden on society, both socially and economically. Recent advances in genomic technologies have linked both diseases to shifts in the oral microbiota – a community of >700 bacterial species that live within the mouth. The development of oral microbiome transplantation draws on the success of fecal microbiome transplantation for the treatment of gut pathologies associated with disease. Many current *in vitro* oral biofilm models have been developed but do not fully capture the complexity of the oral microbiome which is required for successful OMT. To address this, we developed an *in vitro* biofilm system that maintained an oral microbiome with 252 species on average over 14 days. Six human plaque samples were grown in 3D printed flow cells on hydroxyapatite discs using artificial saliva medium (ASM). Biofilm composition and growth were monitored by high throughput sequencing and confocal microscopy/SEM, respectively. While a significant drop in bacterial diversity occurred, up to 291 species were maintained in some flow cells over 14 days with 70% viability grown with ASM. This novel *in vitro*

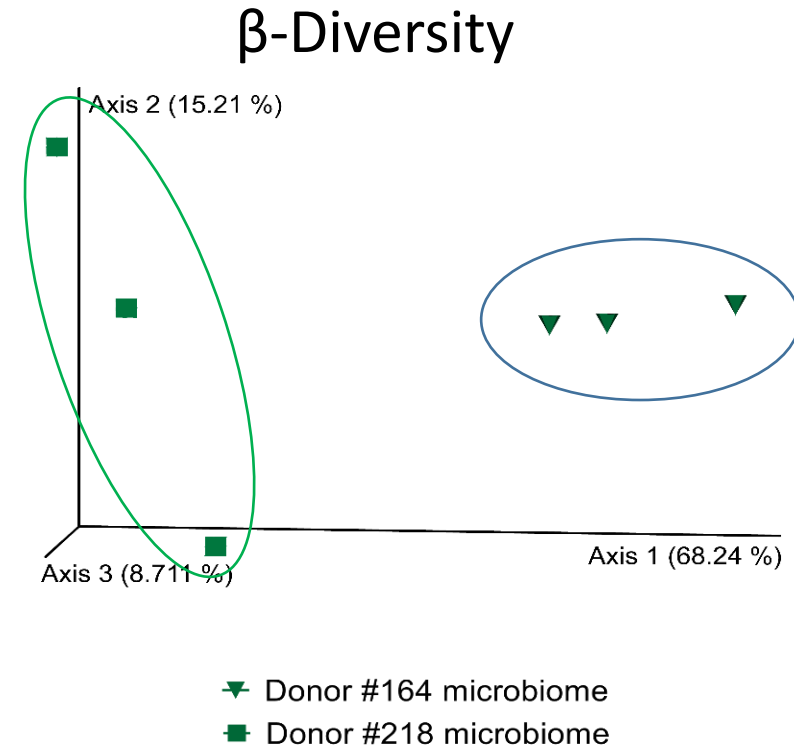
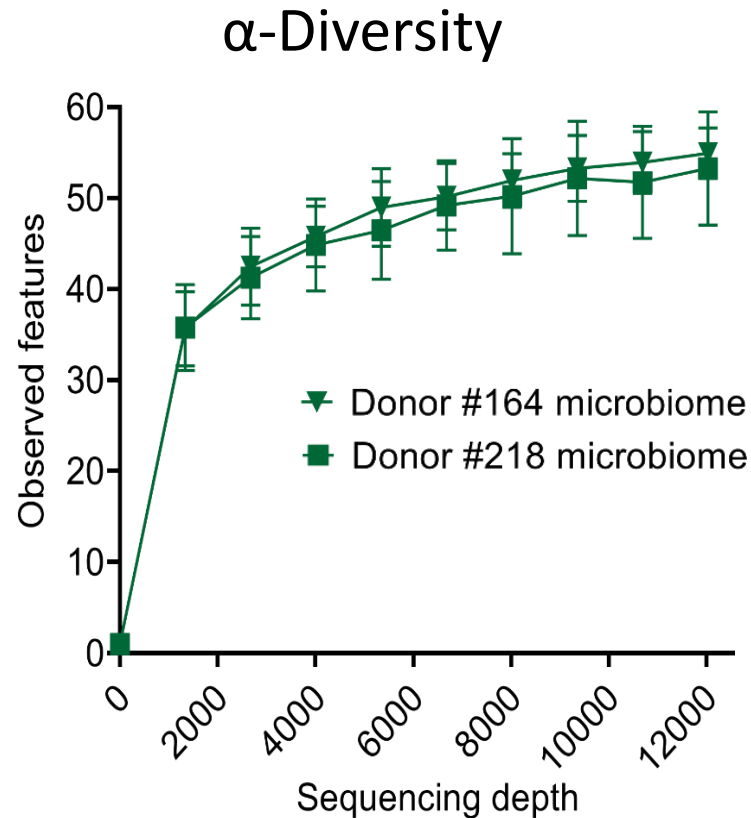
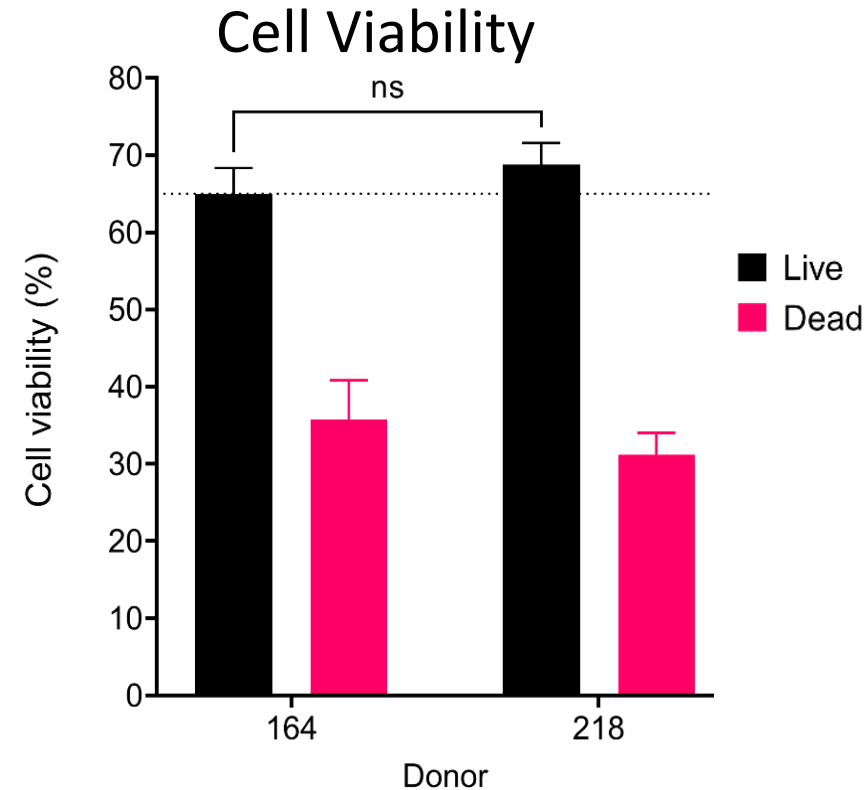
Alpha and Beta diversity explained



Donor microbiome grown in flow cells for 10 Days in ASM



- Cell viability determined using Confocal Laser Scanning Microscopy and Imaris® software n=6
- DNA sequencing libraries generated by amplifying V4 hypervariable region of 16S rRNA gene
- α -diversity calculated using observed features ($p = 0.66$). (ca. 240 taxa maintained over 10 days)
- β -diversity visualised using principal coordinate analysis- significant difference



*** Protocol repeated for Optimal & Sub optimal donor**

Does OMT Work?

**Female Sprague
Dawley Rats
(different litters)
Weaned at Day18
(N = 48)**

**Group 1
Cariogenic Diet
+ CMC. N = 12**

**Group 2
Cariogenic Diet
+ *S. mutans* in CMC
N = 12**

**Group 3
Cariogenic Diet
+ **OMT** in CMC
N = 12**

**Group 4
Cariogenic Diet
OMT + *S. mutans* in CMC
N = 12**

OMT success?
Jaws assessed for caries by:

- Stereo imaging
- CT scanning
- Direct observation

Microbiome changes
16S rRNA amplicon sequencing

OMT safety

- Serum CRP
- Gut inflammation

Otolaryngology (2012) 101:9-14
DOI 10.1007/s10266-012-0091-0

REVIEW ARTICLE

Rodent model in caries research

William H. Bowen

Received: 16 August 2012 / Accepted: 13 October 2012 / Published online: 6 November 2012
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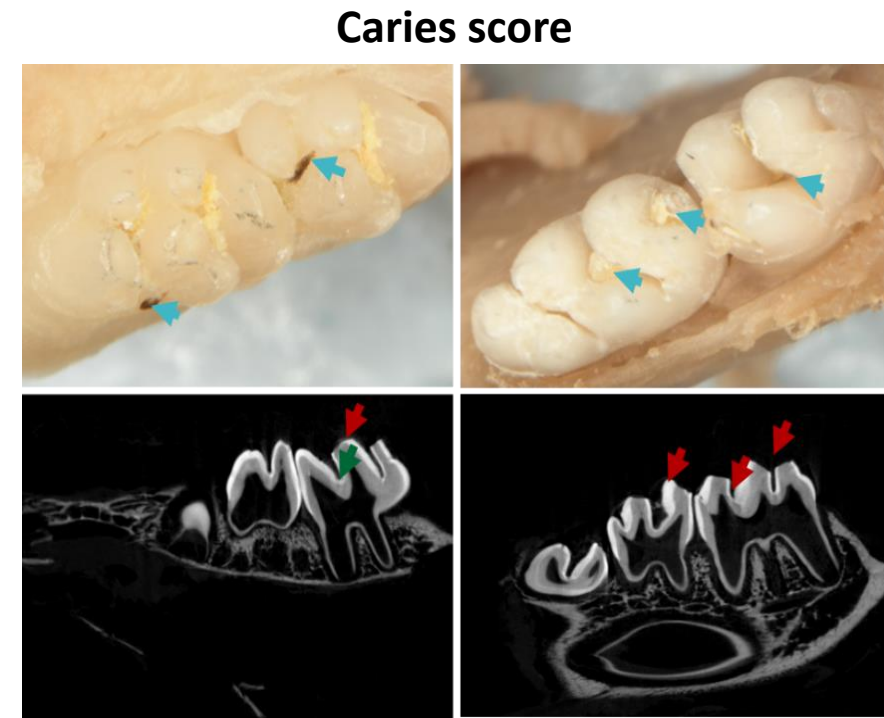
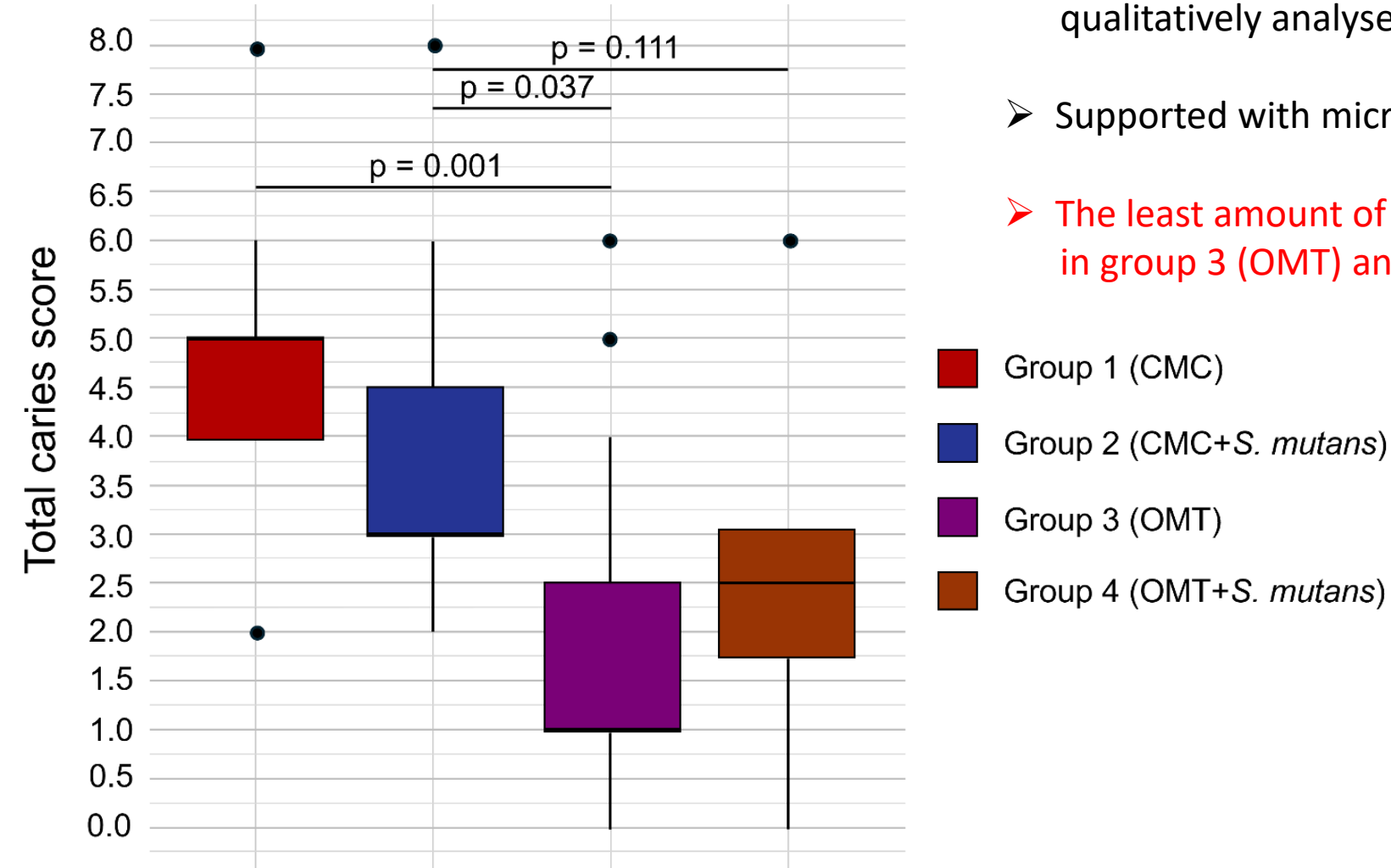
Abstract The rodent model of dental caries has made fundamental contributions to the understanding of the etiology, pathogenesis, and prevention of this disease. In conducting animal-based investigations, important details considered essential for successful outcome are occasionally overlooked. Adequate number of animals of appropriate age should always be included to ensure sufficient statistical power to draw valid conclusions. Attention should be paid to

lack sufficient detail to facilitate evaluation. The rodent model permits a great versatility of design and allows for experimental procedure that may mimic conditions found in humans. A common problem found in reports on caries studies in animals is insufficiency of statistical power that prevents the drawing of valid conclusions.

The development of transgenic animals together with genetic manipulation of microorganisms will facilitate the

Results - Caries in Rats Fed NIH 2000 diet

- Maxillary and mandibular molars of rats from all groups were qualitatively analysed for cavitation, enamel, and dentin loss.
- Supported with micro-CT analysis to quantitate caries severity
- **The least amount of cavitation, enamel and dentin loss was seen in group 3 (OMT) and the most was observed in group 1 (CMC).**



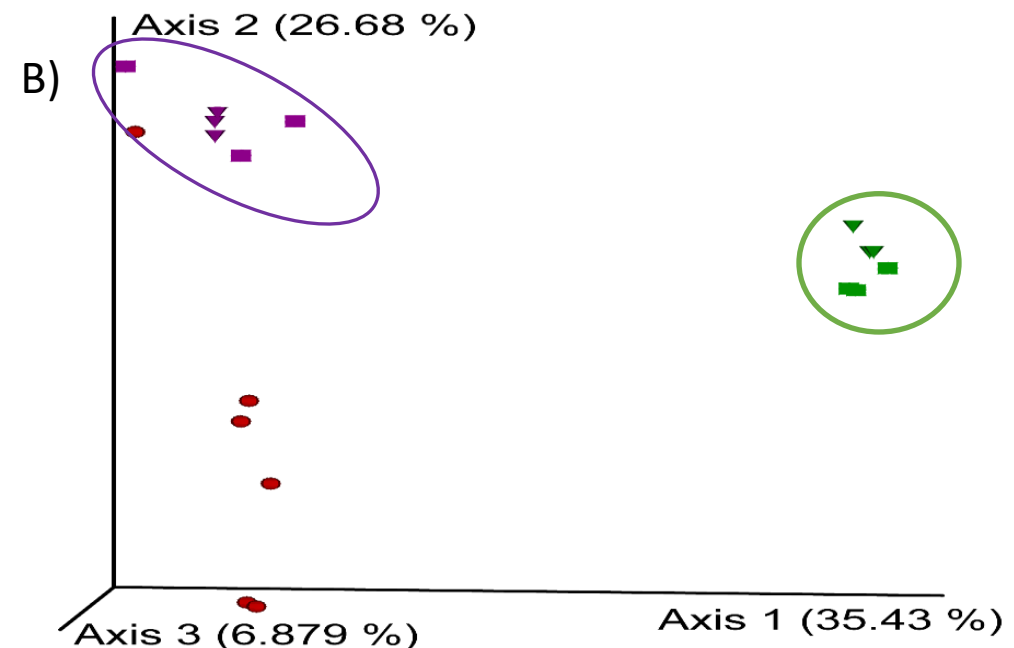
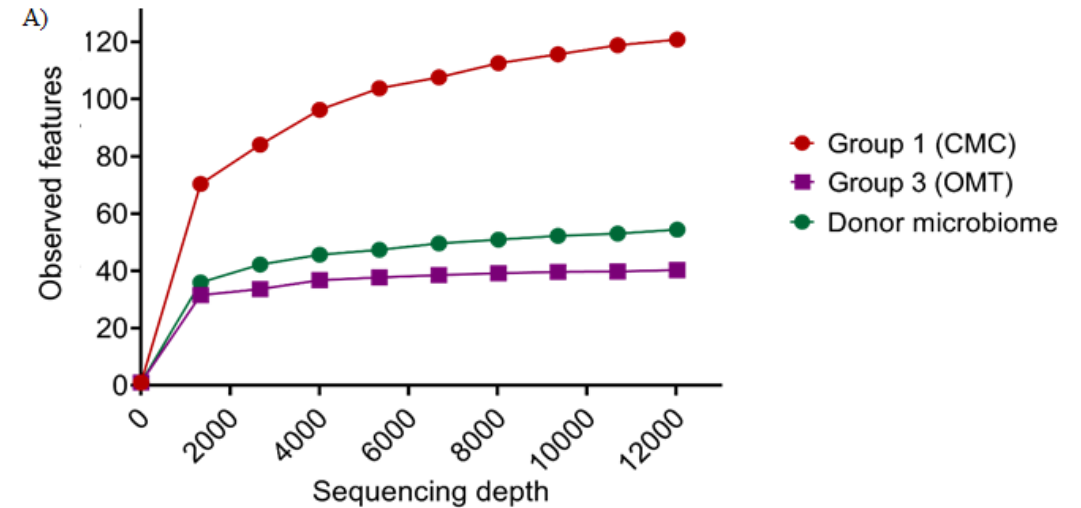
α - and β - diversity in treatment groups pre and post OMT

➤ CMC-only and OMT treatment significantly different α -diversity ($p = 0.01$)

➤ Significant difference in β diversity between control and OMT group

➤ Most abundant taxa following OMT included, Rothia, Streptococci and Muribacter

➤ OMT created a “hybrid” microbiome that was closer to the donor than the rat microbiome.

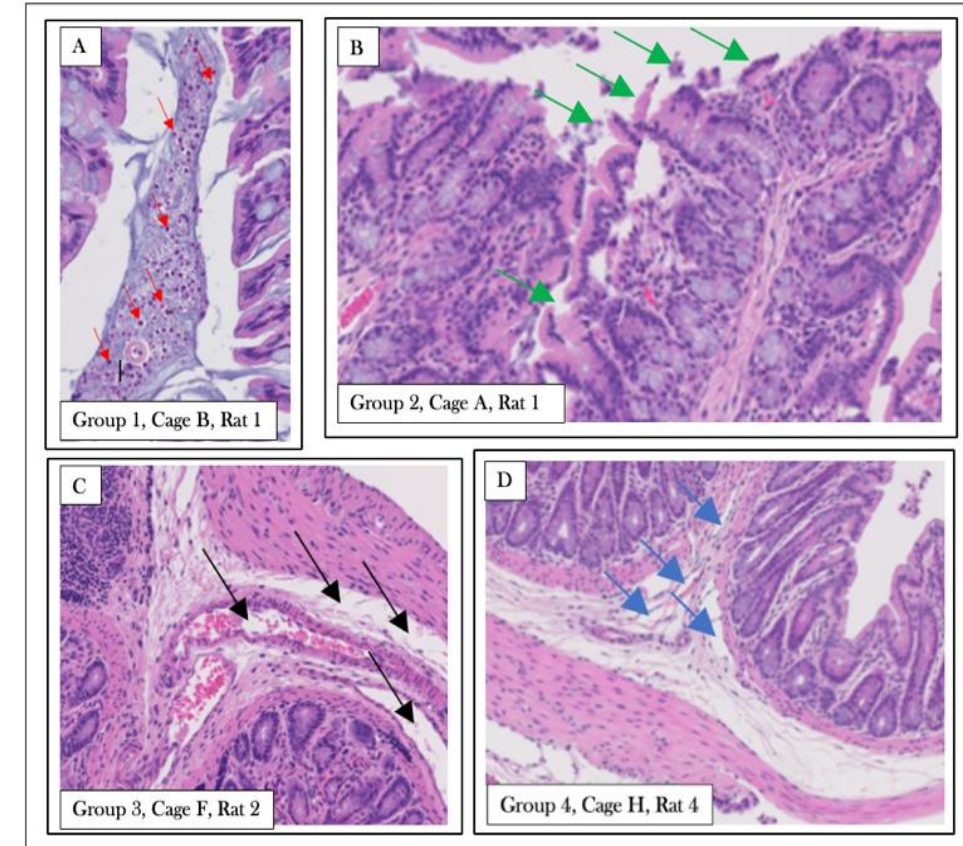
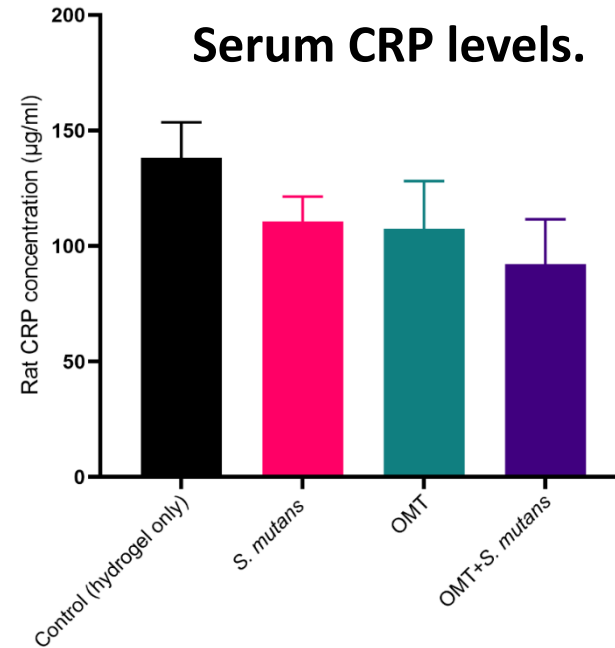
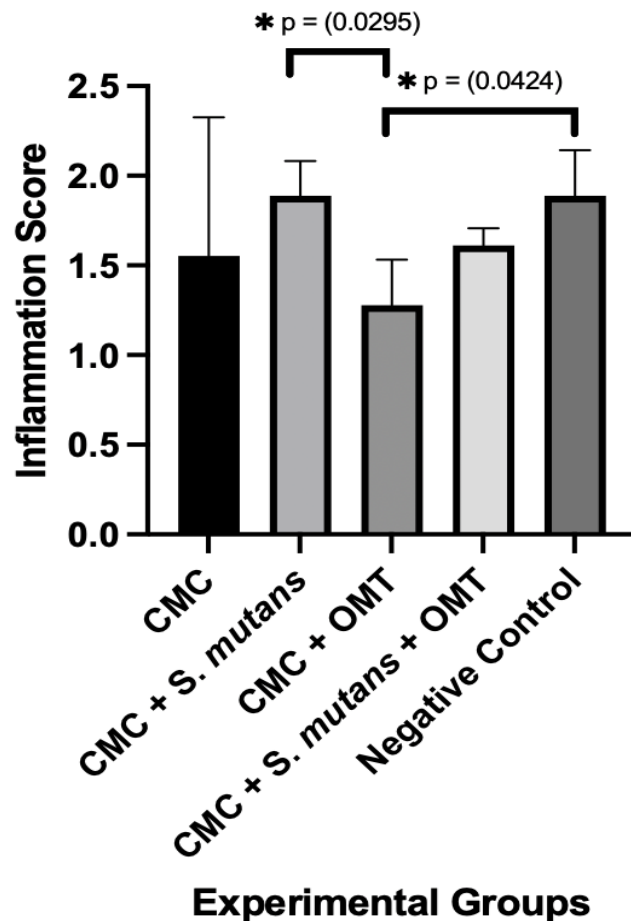


Is OMT safe?

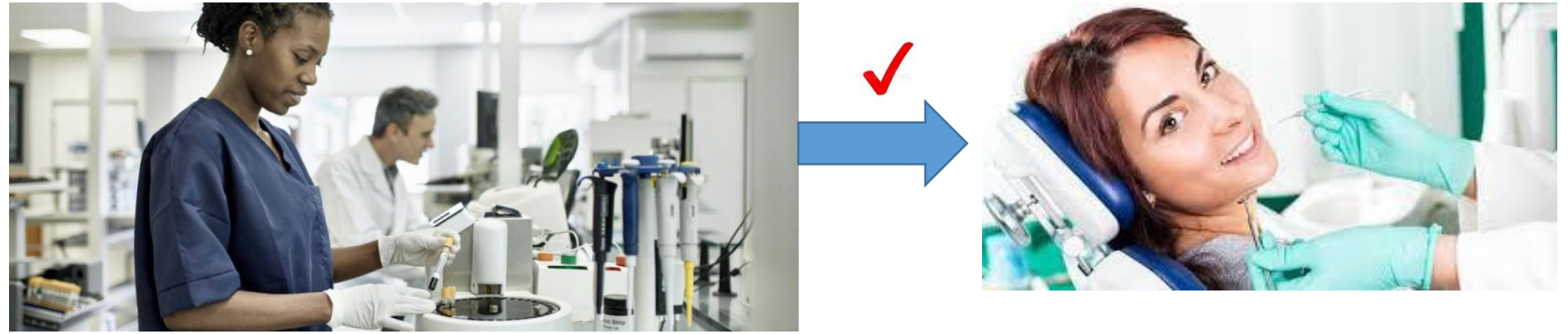
Objective: To observe the effect of OMT on gut inflammation

- Large intestine removed and stained with hematoxylin and eosin.
- Total tissue injury score based on the five histological criteria in the large intestine
- Serum C-reactive protein

Average of all 3 Sites of the Large Intestine



Conclusion



- Growth of the human oral microbiota in a flow cell over 10 days in ASM produced a convergence in α -diversity.
- OMT suppressed dental caries in a rodent model and may prove to be an effective anti-caries treatment.
- Oral Microbiome transplantation significantly shifts β -diversity creating a new hybrid microbiome that is not representative of the donor.
- Continued research including developing human clinical trials.



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University



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