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Functional Dyspepsia Duodenum Mucosal Associated Microbiota: Comparison Between Fresh Biopsy and Historical Formalin-Fixed Paraffin-Embedded Biopsies is Viable

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Introduction

Emerging evidence suggests there is a specific duodenal mucosal associated microbiota (MAM) signature for functional dyspepsia (FD), although study numbers are low and consensus on biomarkers have not been reached. Additionally, we now know that FD is associated with high duodenal eosinophil counts and this site-specific immune response is likely interacting or responding to the MAM.

Aim: As duodenal biopsies for research can be difficult to obtain, we aimed to investigate formalin-fixed paraffin-embedded (FFPE) biopsies, routinely collected for pathology, sequence the MAM and compare them to the MAM profiles of matched RNAlater preserved biopsies.

Methods

Duodenal biopsies (n=4) stored in RNAlater were homogenized for 3 mins at 5,0000 rpm using the Precellys 24 homogenizer after removal from RNAlater solution. The total DNA was then extracted and purified with the Promega Maxwell automated DNA recovery system following manufacturer's instructions. DNA from matched FFPE sections (40 µm) were extracted with Qiagen deparaffinization solution and FFPE DNA extraction kit following manufacturer's instructions. 16S rRNA amplicon gene sequencing was completed targeting the V6-V8 region. Reads were processed with QIIME2/DADA2 and referenced against the SILVA SSU r138 database. Analysis of microbiota diversity was performed in R packages and Calypso. Data was transformed using Cumulative Sum Scaling + log2.

Results

The mean sequencing depth of RNAlater biopsies was 4115 and FFPE was 20170. The alpha diversity was significantly greater for FFPE sequenced samples when compared to whole biopsies (Chao1 P=0.029). The taxonomic composition of the top 20 phyla resulted in fewer "unknown" taxonomic assignments for FFPE samples compared to the matched biopsies.

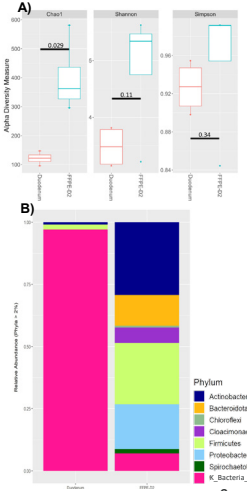

Figure 1. A) Alpha diversity comparisons of Chao1, Shannon, and Simpson indices between whole biopsy extracts and FFPE biopsy sections. A marked increase in diversity is observed across all measures, however, Chao1 is the only significant measure (P= 0.029). **B)** The taxonomic composition for the top 20 phyla of whole biopsy (mean sequencing depth of 4,115 reads) versus FFPE extracted sections (mean sequence depth of 20,170) demonstrates fewer "unknown bacteria" taxonomic assignments for FFPE samples.

Beta diversity differentiation as a result of collection

There was distinct separation of microbial communities when plotted using PCoA and permutational multivariate analysis of variance (PERMANOVA; Adonis function) and ANOSIM Bray-Curtis were also both significant when comparing the MAM of each extraction type (P=0.0297 and 0.023 (R =1), respectively). Further, RDA multivariate analysis demonstrated significant clustering (P=0.024) of FFPE communities compared to the patient matched whole biopsy extracts.

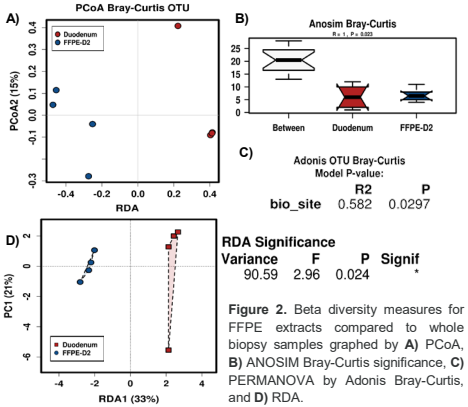

Figure 2. Beta diversity measures for FFPE extracts compared to whole biopsy samples graphed by **A)** PCoA, **B)** ANOSIM Bray-Curtis significance, **C)** PERMANOVA by Adonis Bray-Curtis, and **D)** RDA.

Conclusion

Here we have shown that MAM 16S rRNA amplicon gene sequencing from FFPE tissue sections (tested 2-4yrs old) is a viable option. These results are meaningful for a number of reasons

1. FFPE extracted samples had increased microbial diversity compared to whole biopsy extracts.
2. It demonstrates that single biopsies can be multi-functional (histopathology and viable DNA recovery).
3. The viability of MAM sequence amplification from FFPE provides a tantalising opportunity as the historical catalogue of samples where there were previously no specific biopsies collected for microbial analysis is extensive and would substantially contribute to the integration of FD associated MAM profiles.

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Conflicts of Interest

ECH, KF, GC, SK MM, and SK and have no disclosures. NJT reports personal fees from ARENA Pharmaceuticals, Allakos, Anatara Life Sciences, Bayer AG, Comvita Mānuka Honey, BluMaiden, Glutagen, Intrinsic Medicine, Planet Innovation Progenity Inc., Rose Pharma, and Viscera Labs.

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