

BACKGROUND

- Using traditional methods, acquiring sufficient quantity and quality of DNA from teeth samples can pose significant processing challenges
- Formalin-Fixed Paraffin-Embedded (FFPE) tissue is standard procedure for DNA extraction from bone and soft tissue
- There is a lack of research on microbial DNA extraction from carious primary teeth that have undergone FFPE
- There is also limited research on microbial DNA extraction from freshly extracted, Formalin-Fixed, Macro-dissected, and Pulverized (FFMP) carious primary teeth

PURPOSE

To develop a method for isolation of DNA from FFPE and FFMP carious primary teeth for the ultimate purpose of microbiome assessment.

METHODS

- 20 samples collected (age 3-13)**
 - FFMP carious primary teeth ($N=11$)
 - FFPE carious primary teeth ($N=9$)
- Macro-dissection with Buehler IsoMet precision saw
- Pulverization with liquid nitrogen (2 minutes) and BeadBug3 Microtube homogenizer (4000 rpm)
- QIAGEN QIAamp DNA Mini Kit
- QIAGEN QIAamp DNA FFPE Advanced UNG Kit

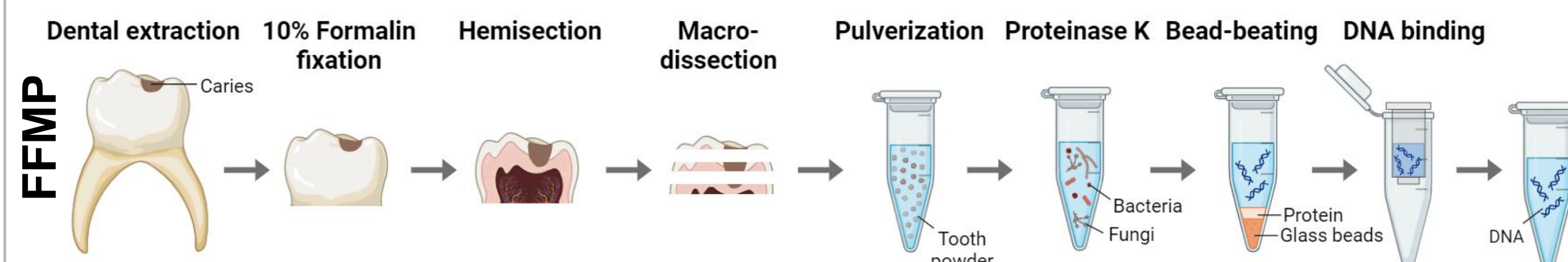


Figure 1. Graphical illustration of Formalin-Fixed Macro-dissected Pulverized (FFMP) DNA extraction steps

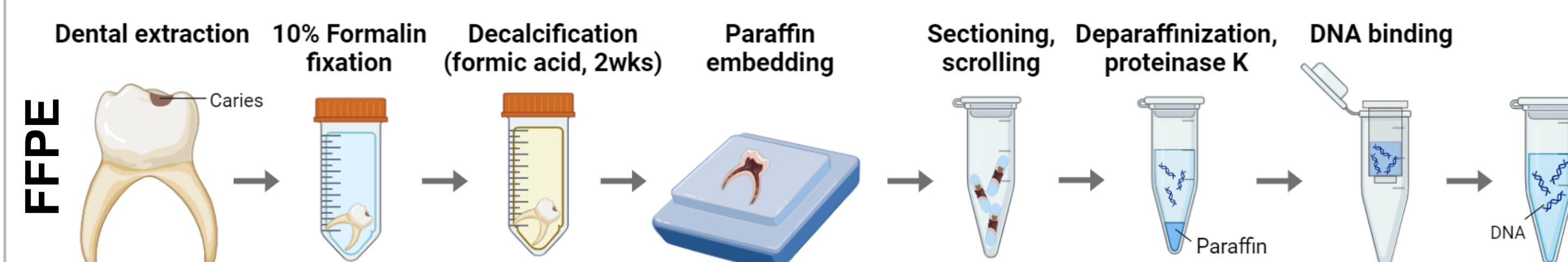


Figure 2. Graphical illustration of Formalin-Fixed Paraffin-Embedded (FFPE) DNA extraction steps

RESULTS

Thin sections of equivalent thickness (200-500um) were reliably created from mineralized primary teeth using an IsoMet precision saw

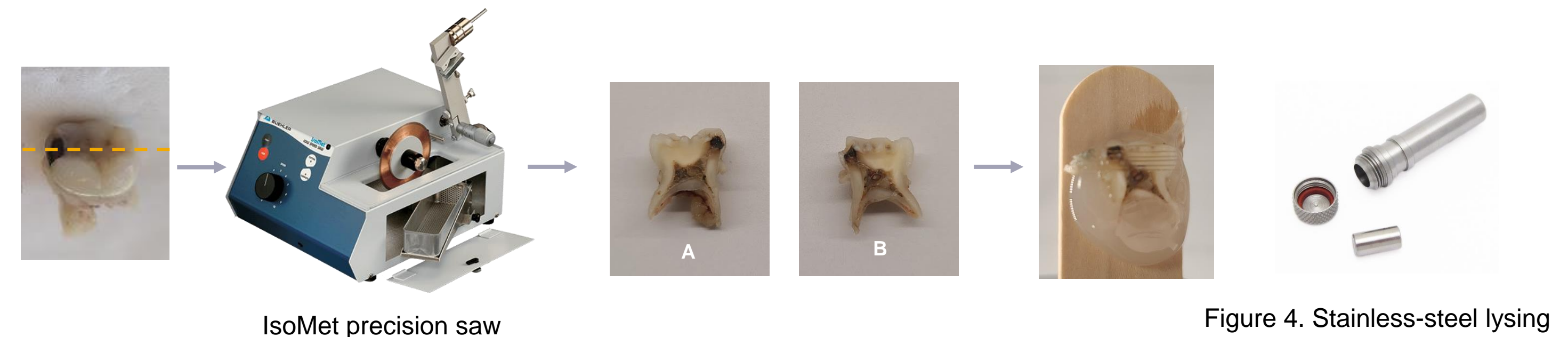


Figure 3. Macro-dissection of a carious primary tooth sample in FFMP method

Figure 4. Stainless-steel lysing tube with cylindrical grinding hammer used for pulverization of each tooth section into powder

DNA was consistently extracted using our developed methods

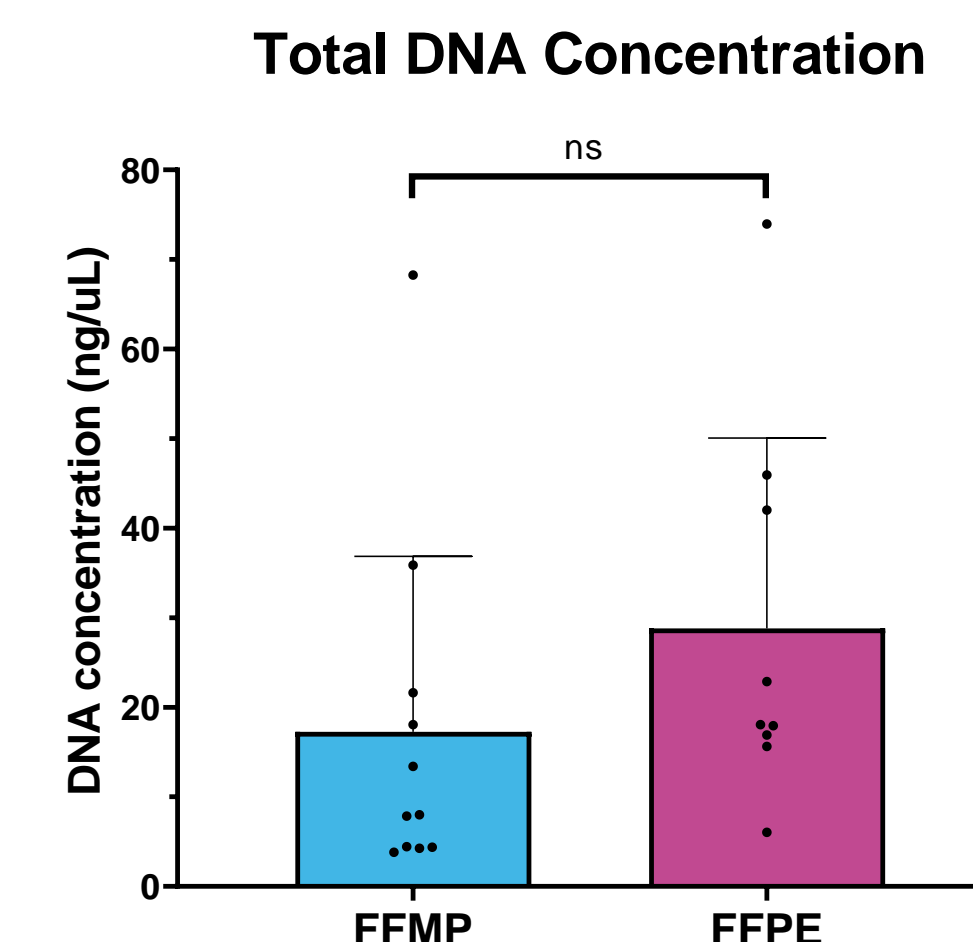


Figure 5. Extracted DNA measured with NanoDrop1000 spectrophotometer. There was no significant difference in the average DNA quantity isolated from FFMP and FFPE samples ($P>.05$, t-test).

Bacterial and fungal DNA were confirmed by qPCR

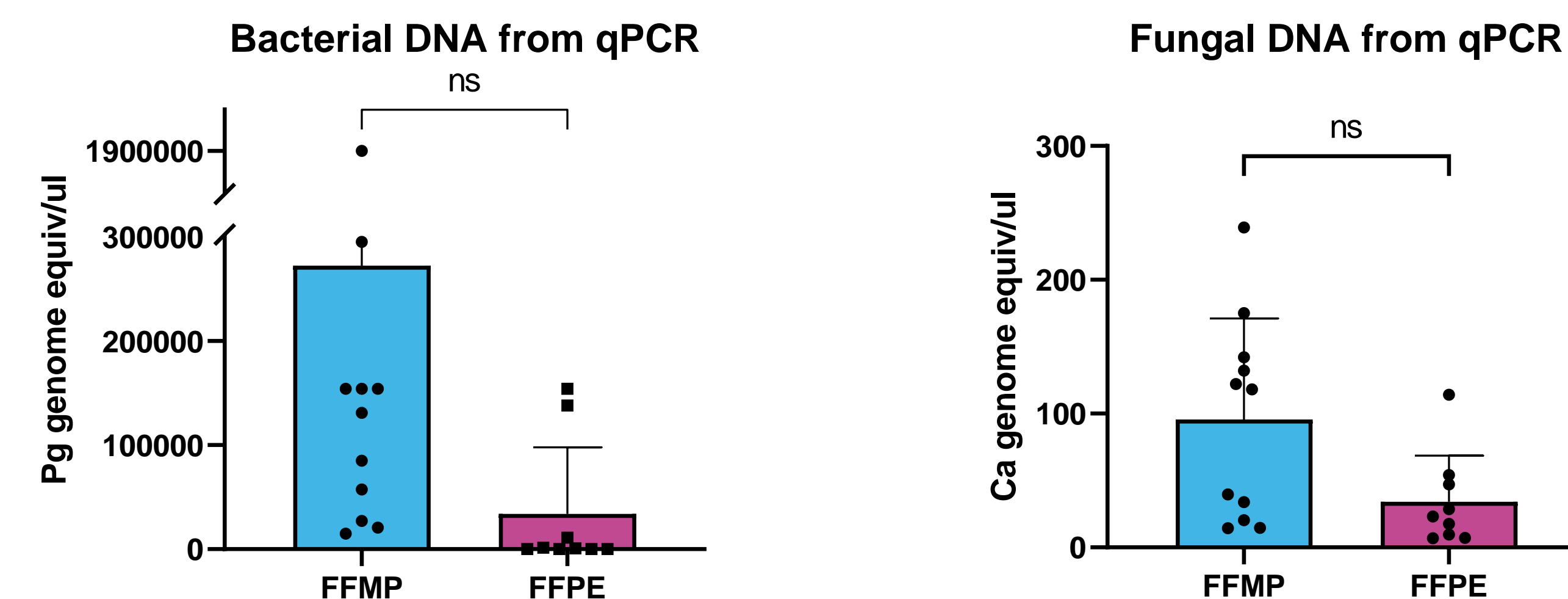


Figure 6. Quantitation of bacterial DNA. Total bacterial DNA was determined with qPCR using *Porphyromonas gingivalis* (Pg) genomic DNA primer as standard. The average bacterial DNA isolated from FFMP and FFPE samples was comparable ($P>.05$, t-test).

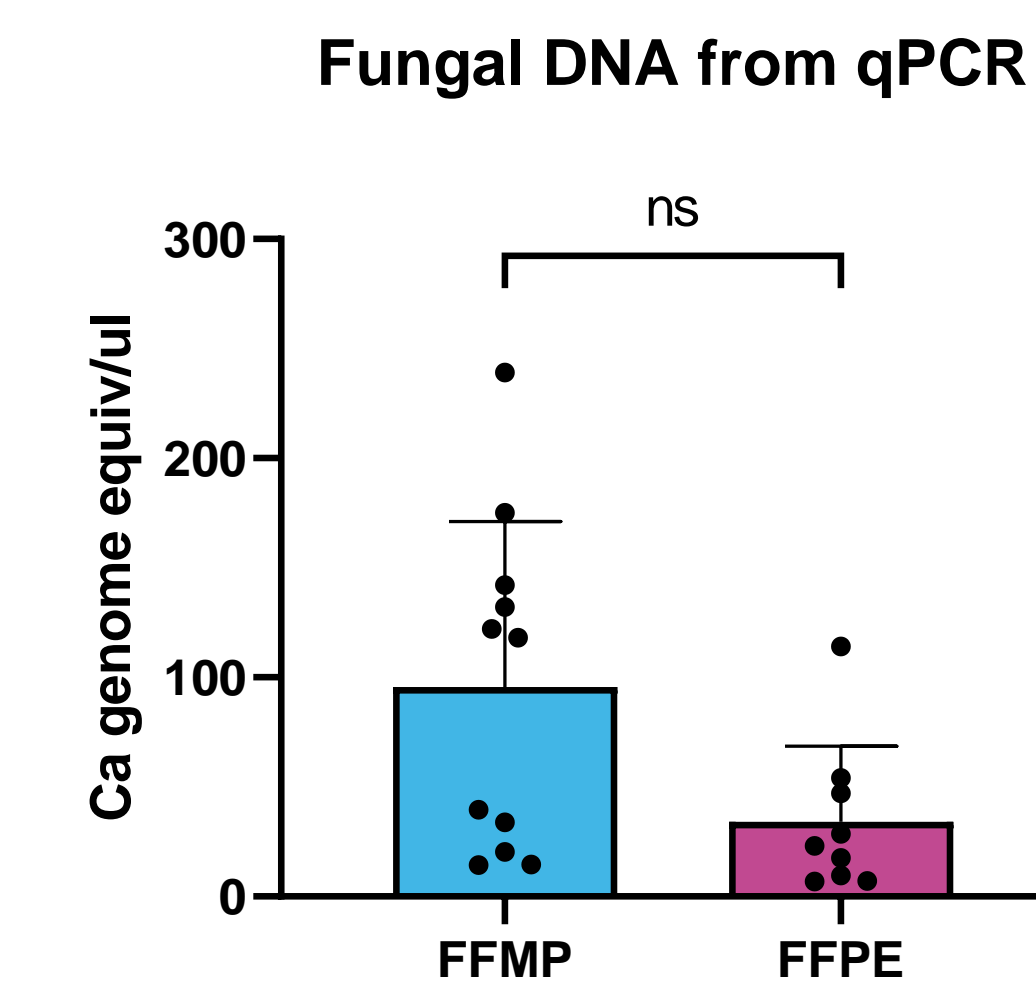


Figure 7. Quantitation of fungal DNA. Total fungal DNA was determined with qPCR using *Candida albicans* (Ca) genomic DNA primer as standard. The average fungal DNA isolated from FFMP and FFPE samples was comparable ($P>.05$, t-test).

CONCLUSION

- This study establishes two novel methods that demonstrate feasible and consistent microbial DNA isolation from carious primary teeth
- DNA can be isolated from carious primary teeth using FFMP and FFPE methods
- Formalin fixation, decalcification, and paraffin embedding do not appear to decrease the DNA yield
- No statistically significant difference in DNA yield was observed between FFMP and FFPE methods

FUTURE DIRECTIONS & IMPLICATIONS

- Future studies include next generation sequencing to compare bacterial and fungal DNA to previously reported communities in carious dentin
- FFMP allows DNA to be isolated from mineralized tissue and does not require a decalcification period
- These novel techniques establish a reliable method to study microbial communities in mineralized dental tissues, which will help to further our understanding of caries pathophysiology and develop therapeutic strategies

KEY

FFPE, Formalin-Fixed Paraffin-Embedded; **FFMP**, Formalin-Fixed, Macro-dissected, and Pulverized; **qPCR**, quantitative polymerase chain reaction

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