

# FadA Modulation Impedes *Fusobacterium nucleatum* Mediated Periodontal Bone Loss

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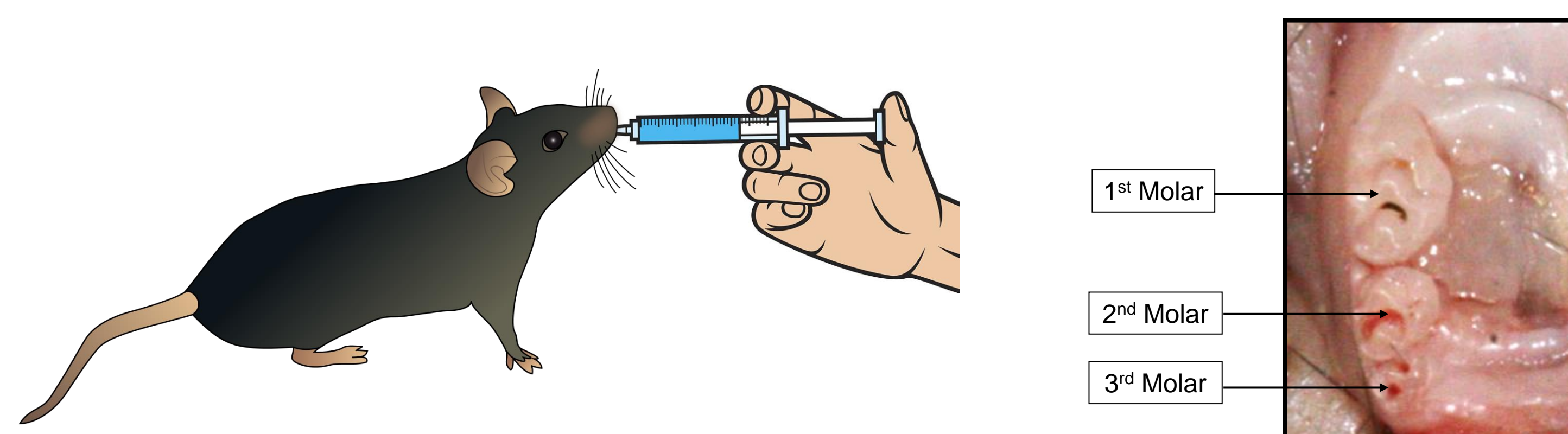
## INTRODUCTION

*Fusobacterium nucleatum* (*F. nucleatum*; *Fn*) is a Gram-negative non-spore forming filamentous anaerobe commonly found in the oral cavity and plays an important role in dental biofilm formation and periodontal disease development. *F. nucleatum* encodes a novel adhesin protein, FadA, which is a potent virulence factor that facilitates the bacterial binding and invasion of host cells. The role of FadA in *F. nucleatum* mediated periodontal tissue destruction, however, is unknown. The aim of this study was to determine whether abrogation of FadA directly impacts *F. nucleatum* mediated periodontal bone loss *in vivo*.

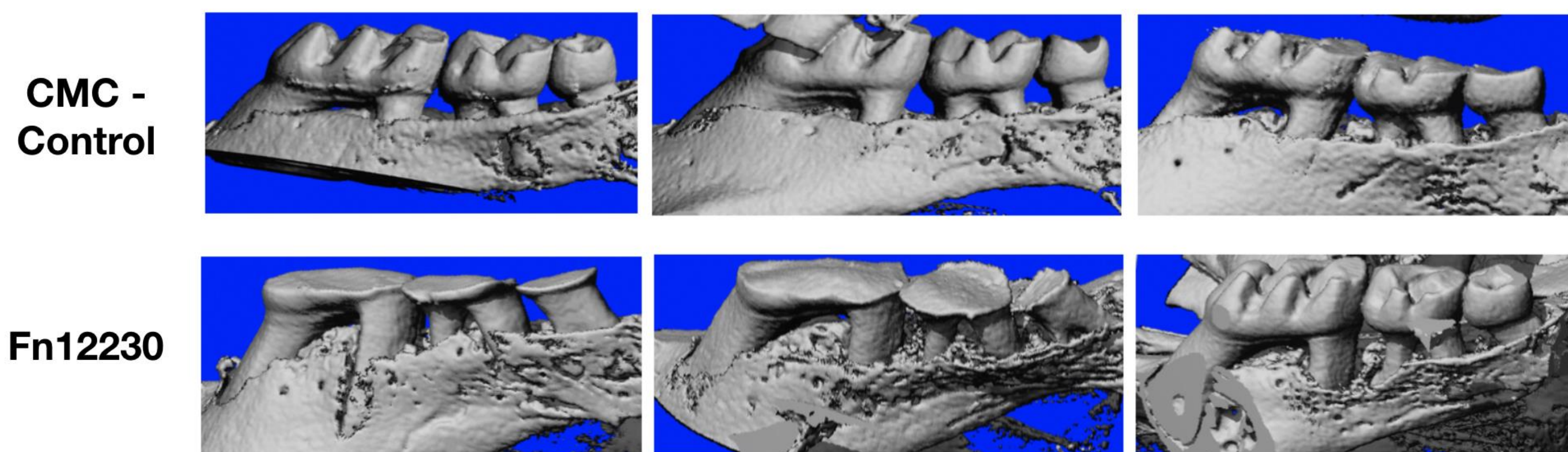
## METHODS

*Fn* 12230 & FadA deletion mutants US1 & lam (1x10<sup>9</sup> CFU) were suspended in carboxymethylcellulose (CMC) and administered orally to 8 week old C57BL/6 mice four times a week for 10 weeks. CMC without *Fn* was also administered as a control. Maxillae were harvested, fixed in 4% paraformaldehyde (PFA) for 24 hours, and stored in 70% ethanol for  $\mu$ CT analysis. Specimens were scanned using a Scanco vivaCT 80 system at 55kVp, 145 $\mu$ A, and 250ms integration time. Grayscale images were reconstructed and CEJ junction measurements were made using ImageJ. Density measurements were calculated using standard Scanco analysis software using the same filter and threshold for the particular isolated regions of interest.

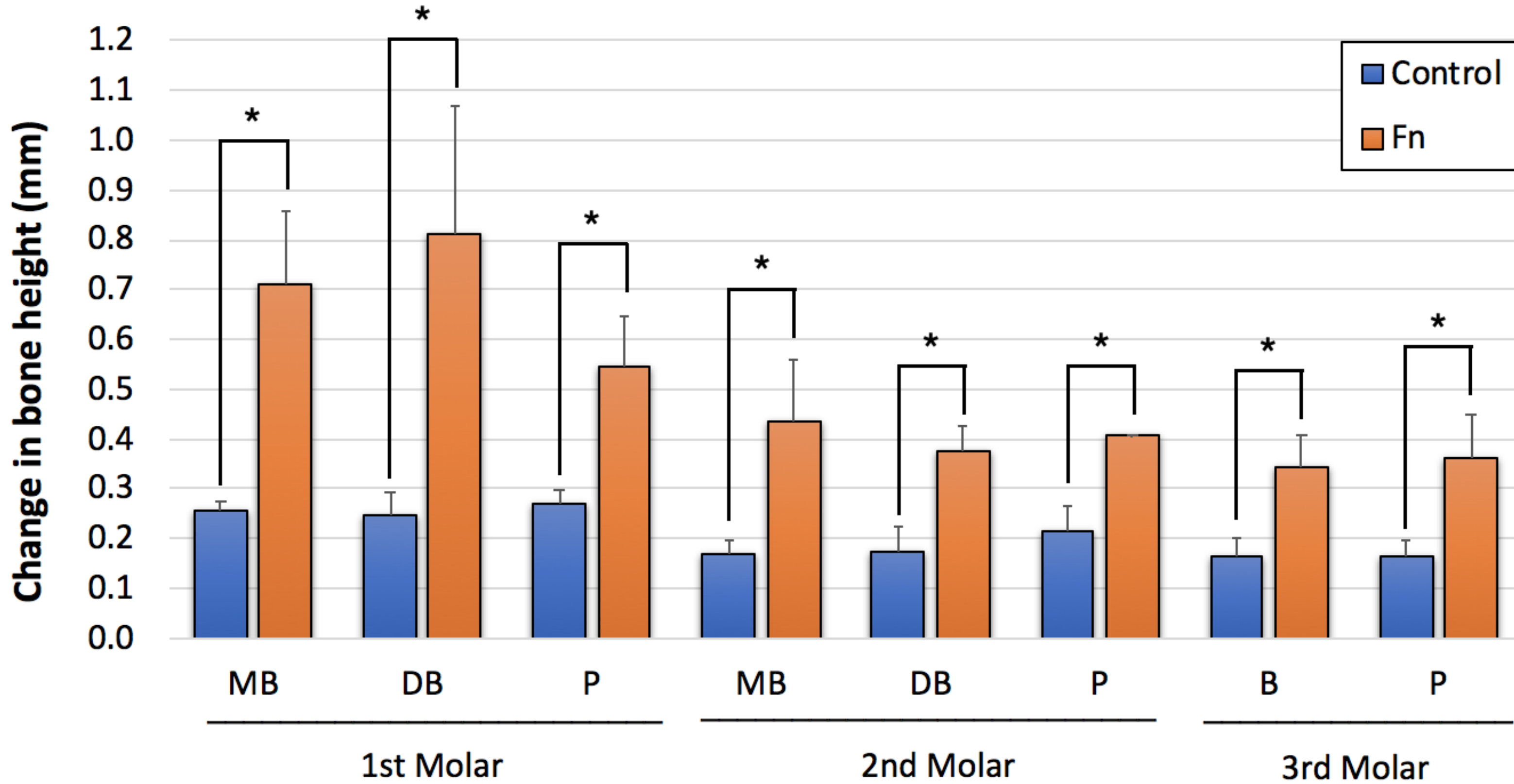
## RESULTS



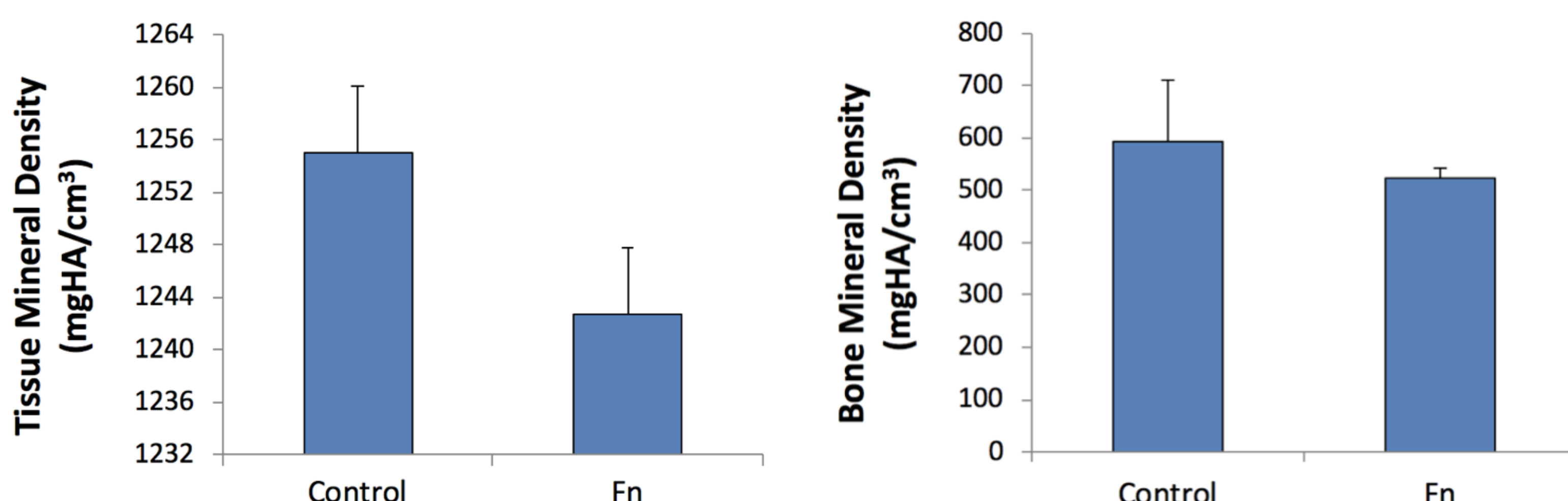
**Figure 1:** *Fn* 12230, US1, and lam (approximately 1x10<sup>9</sup> CFU) resuspended in carboxymethylcellulose (CMC) were orally administered to 8 week old C57BL/6 mice four times a week for 10 weeks. Initially 6 mice were inoculated with CMC alone (3 mice) or *Fn* 12230 (3 mice), and an additional 19 mice were inoculated with CMC (5 mice), *Fn* 12230 (5 mice), US1 (5 mice), and lam (4 mice). Maxillae were harvested and fixed in 4% PFA for 24 hours, then 70% ethanol prior to  $\mu$ CT scanning. Visual inspection confirmed maxillary 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> molars remained intact & maxillae were not damaged during harvesting.



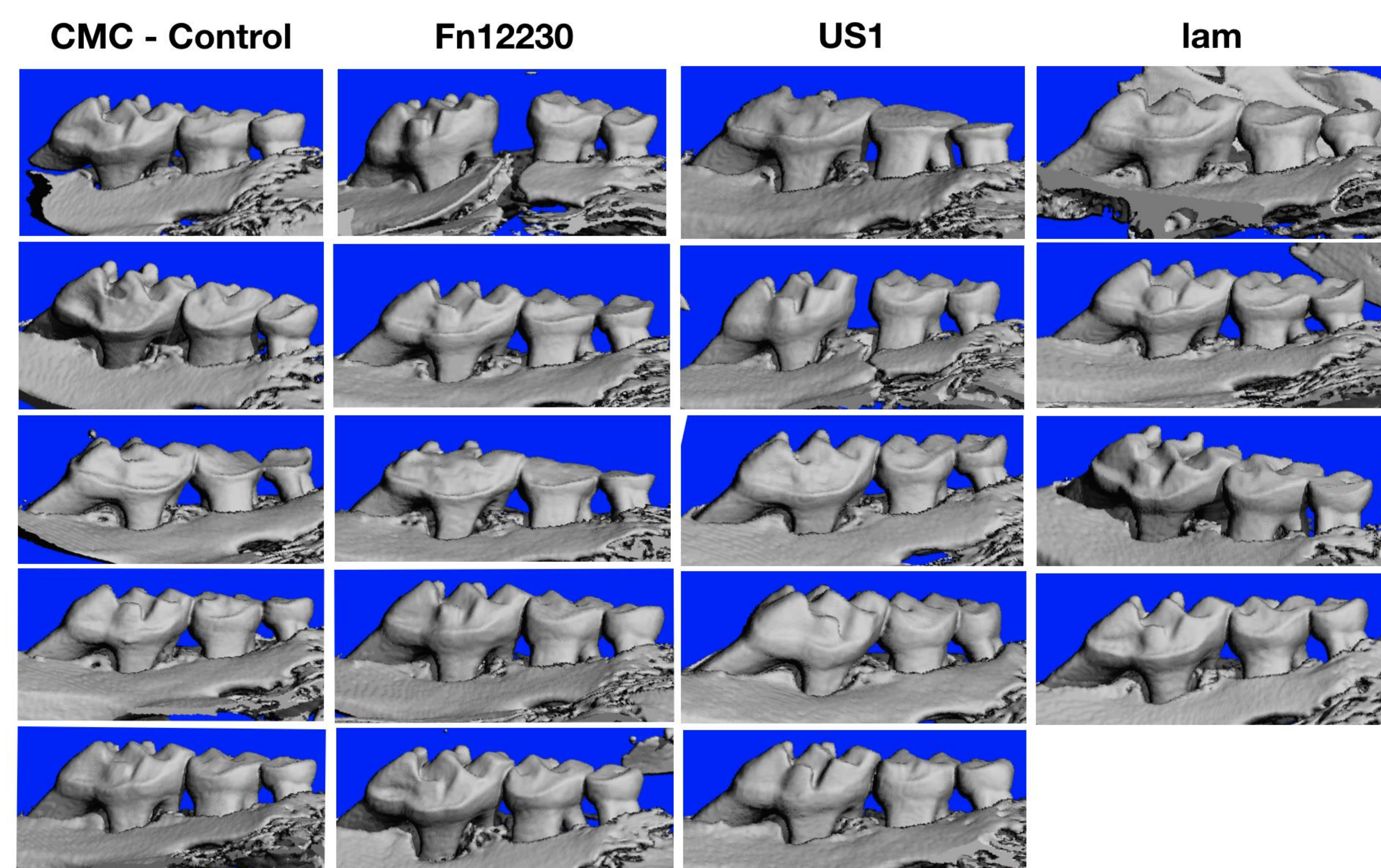
**Figure 2:** Maxillae harvested from three CMC & and three *Fn* 12230 inoculated CB57BL/6 mice were scanned using a Scanco vivaCT 80 system at 55kVp, 145 $\mu$ A, and 250ms integration time. Grayscale images were reconstructed CEJ to alveolar crest measurements were made using ImageJ.



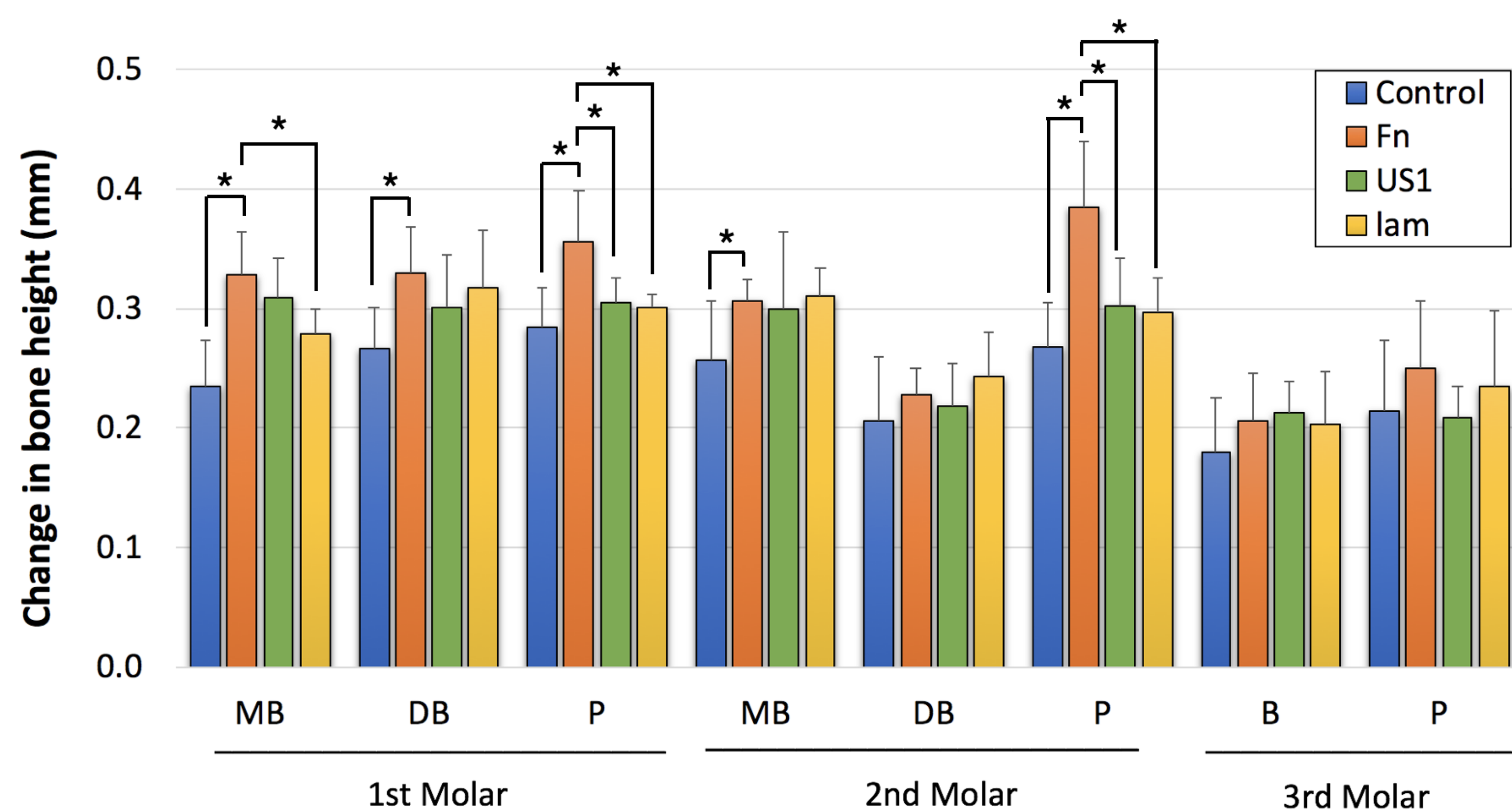
**Figure 3:** ImageJ was used to measure difference in bone height from the cementum enamel junction (CEJ) to the alveolar crest at mesiobuccal (MB), distobuccal (DB), buccal (B), and palatal (P) roots of first, second, and third molars in control and *Fn* inoculated mice. The differences between groups were examined by two-tailed *t*-test and *p* < 0.05 was considered statistically significant.



**Figure 4:** Specimens were scanned using a Scanco vivaCT 80 system (Brüttisellen, Switzerland) using machine settings of 55kVp, 145 $\mu$ A, and 250ms integration time. Density measurements were calculated using standard Scanco analysis software using the same filter and threshold for the particular isolated regions of interest.



**Figure 5:** Maxillae harvested from five CMC, five *Fn* 12230, five US1, and four lam inoculated C57BL/6 mice were scanned using a Scanco vivaCT 80 system at 55kVp, 145 $\mu$ A, and 250ms integration time. Grayscale images were reconstructed CEJ to alveolar crest measurements were made using ImageJ.



**Figure 6:** ImageJ was used to measure difference in bone height from the cementum enamel junction (CEJ) to the alveolar crest at mesiobuccal (MB), distobuccal (DB), buccal (B), and palatal (P) roots of first, second, and third molars in control, *Fn*, US1 and lam inoculated mice. The differences between groups were examined by two-tailed *t*-test and *p* < 0.05 was considered statistically significant.

## CONCLUSIONS

- Inoculation *F. nucleatum* alone is sufficient to induce pathogen mediated periodontal bone loss in C57BL/6 mice
- Deletion or impaired function of FadA adhesin may impede *Fn* mediated periodontitis and subsequent alveolar bone loss

## ACKNOWLEDGEMENTS

This research was funded by Columbia University College of Dental Medicine.