Bone-targeted Alkaline Phosphatase Improves Alveolar Bone Healing in Mice

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Introduction

Alveolar bone loss poses significant clinical challenges for tooth retention and implant placement. While freeze-dried bone allograft (FDBA) is commonly used for alveolar ridge augmentation, it exhibits limitations in resorption rate and healing time. Tissue-nonspecific alkaline phosphatase (TNAP), a key enzyme in biomineralization, has shown promise in enhancing bone regeneration.

Materials and Methods

Following maxillary molar extraction and standardized alveolar defect creation in mice, subjects were assigned to three treatment groups: control (Sham), FDBA with saline (BGS), and FDBA with TNAP-D_{10} (BGT). Healing was assessed using micro-computed tomography (micro-CT), serum alkaline phosphatase levels, histology, and immunohistochemistry, at 14 and 60 days post-procedure (dpp).

Results

At 14 dpp, the BGT group demonstrated significantly greater bone volume fraction (BV/TV), bone mineral density (BMD), and alveolar bone volume compared to BGS and Sham groups. Alveolar bone dimension (height and width) was also significantly more stable in the BGT group. Although BV/TV at 60 dpp showed no significant differences, BGT maintained significantly higher BMD and alveolar bone width stability. Histological and immunohistochemical analyses revealed a significant increase in new bone formation, and greater expression of BSP and OPN in the short-term BGT group. No systemic increase in serum ALP levels was detected.

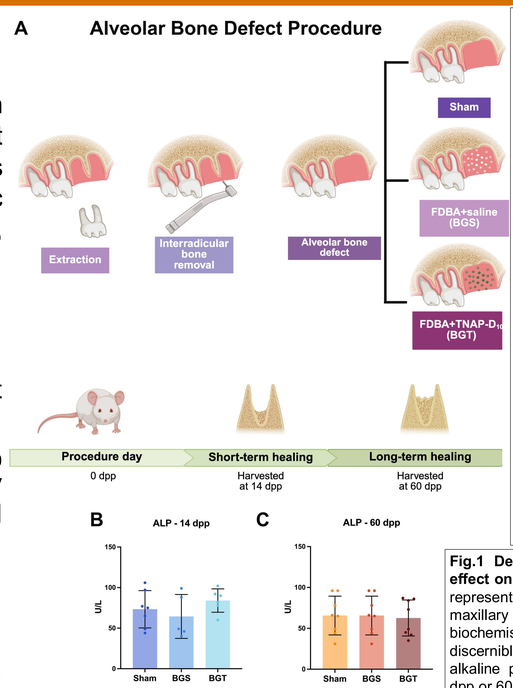

Fig.1 Delivery of TNAP with FDBA has no effect on systemic ALP levels. (A) A schematic representation summarizes creation of the mouse maxillary molar tooth socket defect.. **(B, C)** Blood biochemistry analyses indicate that there are no discernible treatment effects on circulating alkaline phosphatase (ALP) levels at either 14 dpp or 60 dpp.

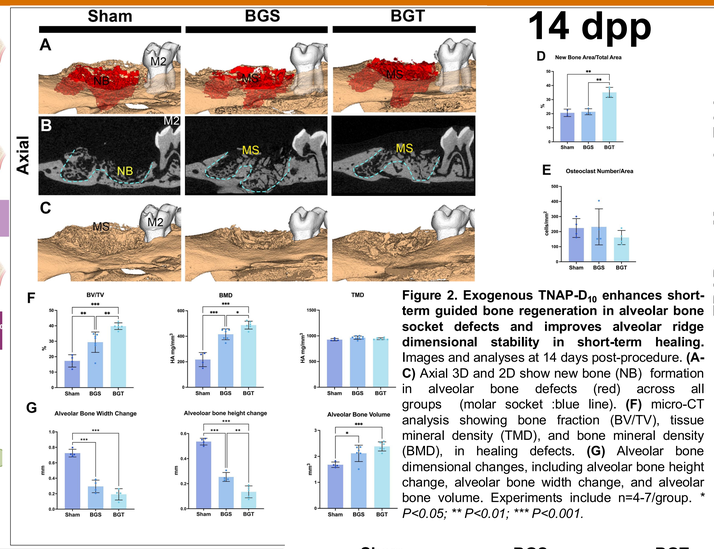

Figure 2. Exogenous TNAP-D_{10} enhances short-term guided bone regeneration in alveolar bone socket defects and improves alveolar ridge dimensional stability in short-term healing. Images and analyses at 14 days post-procedure. **(A-C)** Axial 3D and 2D show new bone (NB) formation in alveolar bone defects (red) across all groups (molar socket :blue line). **(F)** micro-CT analysis showing bone fraction (BV/TV), tissue mineral density (TMD), and bone mineral density (BMD), in healing defects. **(G)** Alveolar bone dimensional changes, including alveolar bone height change, alveolar bone width change, and alveolar bone volume. Experiments include n=4-7/group. * *P<0.05; ** P<0.01; *** P<0.001.*

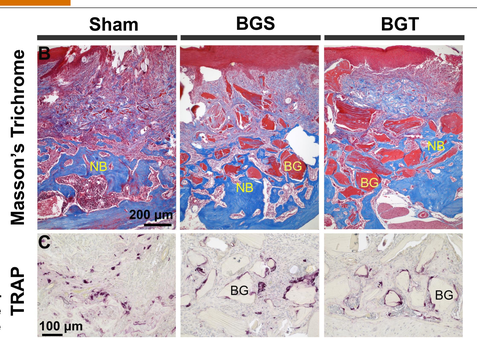

Figure 3. Increased new bone in alveolar bone defects in mice treated with TNAP-D_{10} in short-term healing. Images and analyses at 14 days post-procedure. **(B)** Masson's Trichrome (MT) staining reveals evidence of bone healing and collagen organization within alveolar bone defects, with blue representing bone and red indicating either bone graft particles (BG) or oral epithelium. **(C)** Tartrate-resistant acid phosphatase (TRAP) staining highlights the presence of active osteoclasts within the alveolar bone defects, as indicated by purple-stained cells. **(D)** MT-based quantification of the fraction of new bone formation area to total defect area shows increased levels of new bone in the BGT vs, other groups (n=2-4/group). **(E)** TRAP staining indicates the ratio of osteoclast number to total defect area is not different between experimental groups (n=4/group). ** *P<0.01.* (**NB:** new bone and **BG:** bone graft)

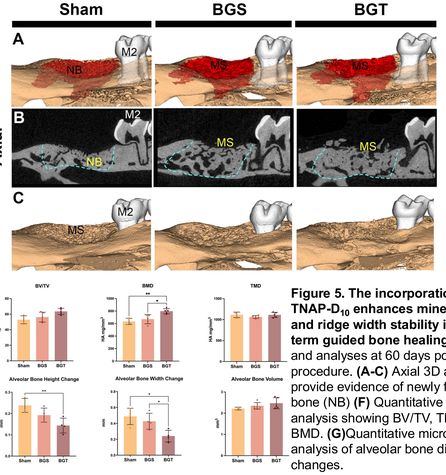

Figure 5. The incorporation of TNAP-D_{10} enhances mineralization and ridge width stability in long-term guided bone healing. Images and analyses at 60 days post-procedure. **(A-C)** Axial 3D and 2D provide evidence of newly formed bone (NB) **(F)** Quantitative micro-CT analysis showing BV/TV, TMD, and BMD. **(G)**Quantitative micro-CT analysis of alveolar bone dimensional changes.

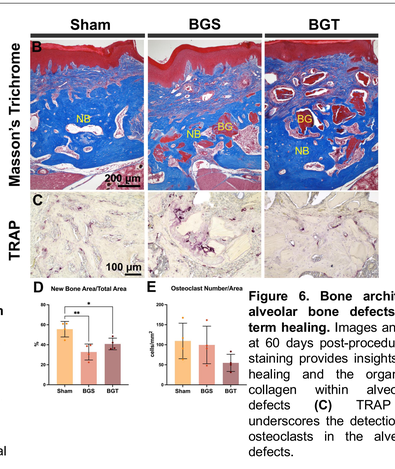

Figure 6. Bone architecture in alveolar bone defects at long-term healing. Images and analyses at 60 days post-procedure. **(B)** MT staining provides insights into bone healing and the organization of collagen within alveolar bone defects **(C)** TRAP staining underscores the detection of active osteoclasts in the alveolar bone defects.

Conclusion

The adjunctive use of TNAP-D_{10} with FDBA significantly enhances early-stage alveolar bone regeneration and dimensional stability. While long-term volumetric gains were not observed, sustained improvements in mineral density and bone preservation support TNAP-D_{10} as a promising biologic for guided bone regeneration. Further studies are warranted to optimize dosing and assess clinical translatability.