Engineering Bacteroides thetaiotaomicron for Targeted Osteocalcin Delivery via Extracellular Vesicles to Treat Osteoporosis

Bellarmine Team A
Aniruddha Janakiraman, Kasra Rategh, Arjav Shah, Maanav Balan | Mentor: Snigdha Jagarlapudi

Problem

Osteoporosis is a systemic bone disorder characterized by **reduced bone mineral density** and an increased susceptibility to bone fractures. It results from an imbalance between **bone resorption** by osteoclasts and **bone formation** by osteoblasts, leading to increased bone deterioration.

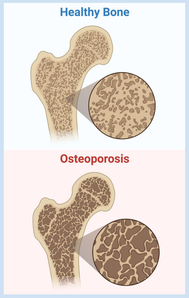


Figure 1.

Osteoporosis affects **200 million people worldwide**, with 80% of U.S. cases in women—often due to estrogen loss during menopause—and causes fractures in 1 in 2 women and 1 in 4 men over 50.

We aim to engineer *Bacteroides thetaiotaomicron* to counter osteoporosis.

Pathophysiology

Osteoporosis occurs when there is an **dysbiosis of gut microbiota**, specifically of Treg and Th17 cells which promote the differentiation of bone marrow STEM cells into osteoblasts and osteoclasts respectively.

Osteocalcin is a key bone matrix protein hormone secreted by osteoblasts. When activated, osteocalcin binds to calcium and hydroxyapatite crystals (key bone mineral) to **strengthen bones.**

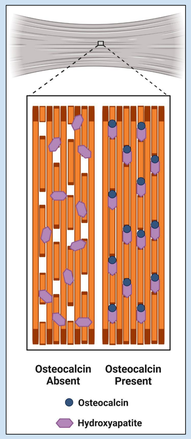


Figure 2. *Diagram Created with Biorender*

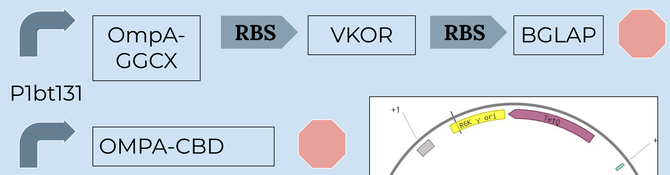
Previous Work

Current treatments are primarily anti-resorptive agents, that inhibit osteoclast differentiation.

Disadvantages		
Denosumab	**Shared**	**Bisphosphonates**
Requires indefinite continual use	Long-term safety concerns	Gastrointestinal side effects
Increases potential for hypocalcemia	Atypical Femoral Fractures	Strict administration guidelines
Rebound effect	Cure not permanent	Slow Onset of Action

Proposed Solution

Plasmid Design



BGLAP: Osteocalcin synthesis
GGCX: Gamma-glutamyl carboxylase
VKOR: Vitamin K hydroquinone reductase
CBD: Collagen-binding domains; target bone extracellular matrix
OMPA: BEV surface protein

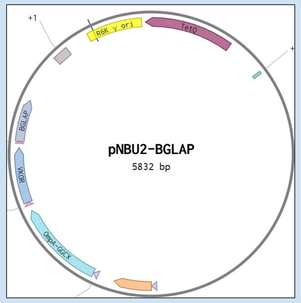


Figure 3. *Plasmid Design made with Benchling*

GGCX helps activate osteocalcin by adding carboxyl groups, allowing it to bind to bone. Vitamin K is needed for osteocalcin activation, and VKOR helps regenerate vitamin K in the bone marrow.

A second cassette includes tags like OmpA to help package osteocalcin into bacterial vesicles, while collagen-binding domains (CBDs) direct it to bone-resorbing cells, improving treatment effectiveness.

Plasmid Engineering and Delivery:

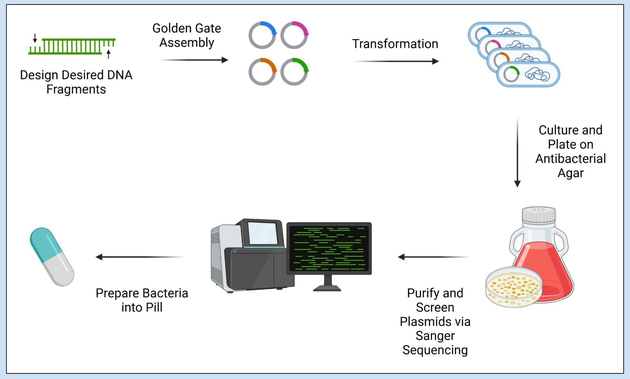


Figure 4. *Workflow Created with Biorender*

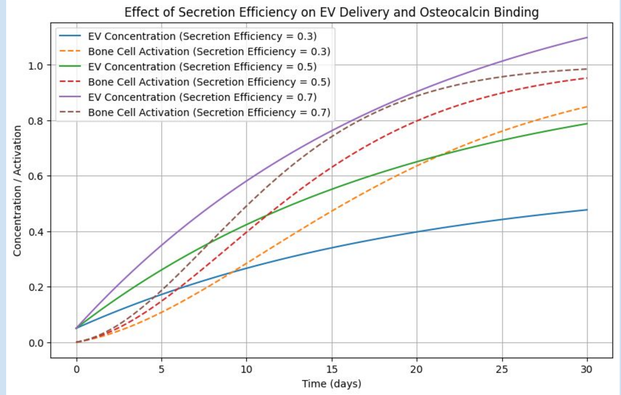
Standard molecular cloning workflow using golden gate assembly will be used to engineer desired constructs into B. thetaiotaomicron. Successful strains will be packaged an oral pill for effective delivery of the therapeutic.

The engineered B. thetaiotaomicron colonizes the gut and produces osteocalcin, which is loaded into BEVs and delivered to the bone matrix. Osteocalcin then binds to calcium and hydroxyapatite, promoting bone mineralization and slowing osteoporosis progression.

Computer Modeling:

Using Python, we can simulate how different variables affect our therapy's effectiveness. For example, by utilizing differential equations, we can predict the effect of binding, transportation, and secretion efficiency on EV concentration and bone cell activation.

Figure 5. *Graph from our python simulation displaying what results different values of secretion efficiency have on EV Delivery and Osteocalcin Binding*



Once we have experimental data, we can integrate these values into our model to quantify their impact. Using the simulated data, we could optimize our plasmid accordingly.

Additionally, the simulation can enable us to explore more complex interactions, which could be important considerations for future work.

Testing & Benchmarking

Osteocalcin Production	Extracellular Vesicle (EV) Production	Binding ability between Osteocalcin and Calcium Ions
ELISA: uses antibodies to detect and quantify levels of osteocalcin in culture media	Size-Exclusion Chromatography: isolates EVs to ensure our bacteria is producing EVS	Binding Assays: to test and optimize the ability of osteocalcin and calcium ions to bind
Western Blot: detects osteocalcin in cell lysates using certain antibodies	Nanoparticle Tracking Analysis: measures and quantifies EV size and concentration	Surface Plasmon Resonance: calcium ions flow over an immobilized osteocalcin, detecting binding

Ethical Considerations

FDA testing
Preclinical and clinical testing + FDA approval are needed for safety reasons

Genetically modified Microorganisms
Potential risks of HGT (Horizontal gene transfer) within the gut microbiome

Cost and Accessibility
Ensuring the pill follows the 2 A's: accessibility and affordability; especially for people that need it the most, such as postmenopausal women.

Technical Limitations

Gene Design Challenges
limited control over transport efficiency; errors with drug function are up to chance; expression of human genes in bacteria system

↓

Delivery Challenges
Stability of EVs, predicting off target effects; EV sensitivity to environmental changes

↓

Implementation Challenges
Large scale testing (in vivo and in vitro); Cost and Accessibility

References & Acknowledgements

Alam, M. Faiyaz, et al. "Osteocalcin, a Promising Marker of Osteoporosis: Evaluation in Post-Menopausal Females with Osteoporosis." *International Journal of Advances in Medicine*, www.ijmedicine.com/index.php/ijam/article/view/2030. Accessed 15 Mar. 2025.

Bone Health and Osteoporosis Foundation. "Osteoporosis Fast Facts." *Osteoporosis Fast Facts*, www.bonehealthandosteoporosis.org/wp-content/uploads/Osteoporosis-Fast-Facts-2.pdf. Accessed 16 Mar. 2025.

Cooke, M. J., Phillips, S. R., Shah, D. S., Athey, D., Lakey, J. H., & Przyborski, S. A. "Enhanced Cell Attachment Using a Novel Cell Culture Surface Presenting Functional Domains from Extracellular Matrix Proteins." *Cytotechnology*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/19002844/. Accessed 15 Mar. 2025.

Ferron, M., Lacombe, J., Germain, A., Oury, F., & Karsenty, G. "GGCX and VKORC1 Inhibit Osteocalcin Endocrine Functions." *The Journal of Cell Biology*, U.S. National Library of Medicine, 16 Mar. 2015, pubmed.ncbi.nlm.nih.gov/25753038/.

García-Bayona, L. E., & Comstock, L. E. "Streamlined Genetic Manipulation of Diverse Bacteroides and Parabacteroides Isolates from the Human Gut Microbiota." *mBio*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/31409684/. Accessed 15 Mar. 2025.

Komori, Toshihisa, et al. "What Is the Function of Osteocalcin?" *Journal of Oral Biosciences*, Elsevier, 12 June 2020, www.sciencedirect.com/science/article/abs/pii/S1349007920300542.

Lyu, Z., Hu, Y., Guo, Y., & Liu, D. "Modulation of Bone Remodeling by the Gut Microbiota: A New Therapy for Osteoporosis." *Bone Research*, U.S. National Library of Medicine, 9 June 2023, pubmed.ncbi.nlm.nih.gov/37296111/.

Mayo Clinic. "Osteoporosis." *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 24 Feb. 2024, www.mayoclinic.org/diseases-conditions/osteoporosis/diagnosis-treatment/drc-20351974.

Office of Public Outreach and Communication. "Home." *Newsroom*, 12 May 2020, sebsnjaesnews.rutgers.edu/2020/05/americans-urged-to-increase-their-knowledge-of-bone-health-during-osteoporosis-month-in-the-u-s/.

Simmons, Rick. "Understanding Bone Resorption: Aquidneck Chiropractic." *Aquidneck Chiropractic*, 4 Dec. 2018, aquidneckchiropractic.com/understanding-bone-resorption/.

Zoch, Meredith L., et al. "New Insights into the Biology of Osteocalcin." *Bone*, U.S. National Library of Medicine, Jan. 2016, pmc.ncbi.nlm.nih.gov/articles/PMC4670816/.

Thank you to BioEHSC and ASUC for their support.