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Bone characterization in the treatment of Hypophosphatasia with mesenchymal stem cells

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We present the use of second-harmonic generation (SHG) images to characterize bone morphology using Hypophosphatasia (HPP) as a disease model. Hypophosphatasia (HPP) is a rare genetic disorder caused by mutations to the tissue-non-specific alkaline phosphatase (ALP) gene. Diminished ALP activity prevents the enzyme from dephosphorylating inorganic pyrophosphate (PPi), a potent inhibitor of mineralization, resulting in disarticulated collagen and porous bones. Current treatments only alleviate symptoms in the long bones of patients with HPP and do not address premature loss of teeth and craniosynostosis. A promising treatment is mesenchymal stem cell (MSC) therapy, which has been used in clinical studies along with myeloablation and full bone marrow transplants. However, many patients in need do not qualify for bone marrow transplants as they are too sick for such a harsh, risky procedure. While it is established that HPP reduces mineralization in bone, the effect of HPP on other parameters of bone formation, such as bone microarchitecture, is unknown. First, a method for describing pores had to be established. SHG images were used to examine the collagen microstructure in cranial bones of both healthy and HPP mice. Image J was then used to analyze characteristics of bone such as pore size, number, and spacing. As the surface of the skull is curved, the image was first flattened by removing tilt. Next, a sub-region of interest was selected and a projection was created. This image was processed using several different methods and then various thresholds were added to each processed image. Automated pore counts on a small subset of data were compared to manual counts to optimize the way pores are analyzed. The percentage of manually counted pores that was automatically counted and their standard deviation were both factors in choosing a method. It was determined that using sequential image processing was the best protocol for describing pores.In future studies, this method of describing pores will be used to assess the impact of MSCs in their local environment. We predict that bones will have smaller, fewer pores and denser collagen fibers when a mouse is treated with MSC therapy. This data will be used to determine the effectiveness of MSC therapy for HPP and will establish SHG as a means for characterizing bone morphology for bone diseases.