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Development of a protocol for the maintenance and mineralization of osteoblasts from *oim* mice

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Osteogenesis imperfecta (OI) is a disease of type I collagen whose hallmark is extreme bone fragility manifested by numerous fractures throughout life. OI is usually caused by decreased amounts of type I collagen and/or the production of abnormal collagen, leading to biomechanically impaired extracellular matrix. Osteoblasts are cells that build the matrix constituents of bone by secreting collagen, which, along with hydroxyapatite crystals, forms the composite structure of bone. It is unclear how osteoblasts respond to a structurally compromised matrix at a cellular level. This project is a pilot study to explore how osteoblasts from the osteogenesis imperfecta mouse (*oim*) model differ from wildtype osteoblasts, and how these differences contribute to the structurally inferior bone matrix of *oim* mice. Because there is no established protocol for growing and mineralizing *oim* osteoblasts in tissue culture, the main focus of my summer research has been developing such a protocol. Osteoblasts were harvested from calvaria taken from *oim* and wildtype mice at three to four days of age using a trypsin-collagenase solution. The cells were then grown in α -MEM in 12-well plates and encouraged to mineralize using β -glycerol phosphate and ascorbic acid. Because the outcome measures are protein quantification, fibroblast cultures have been used to help develop the protocols for measuring hydroxyproline, an amino acid unique to collagen, as well as western blotting of type I collagen. While the protocol for growing and mineralizing osteoblasts is still in development, we have finalized our harvesting and plating procedures, allowing to us effectively grow both wildtype and *oim* osteoblasts in culture.

