

**Use of immortal mouse technology to establish two osteoblast-like cell lines that utilize distinct mineralization processes while expressing Dmp1-GFP**

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The mechanisms that initiate and regulate woven and lamellar bone mineralization remain undefined. To further understand bone mineralization mechanisms, cell models were established from transgenic mice expressing GFP driven by the Dmp1 promoter. The cells were immortalized by crossing these mice with the "Immortomouse," which overexpresses a  $\gamma$ IFN-inducible, temperature-sensitive SV40 large T-antigen. Immortomouse-derived cells are conditionally immortalized at 33°C in the presence of  $\gamma$ IFN and resume their original phenotype at 37°C in the absence of  $\gamma$ IFN. Sequential collagenase-EDTA digestions from 3-week-old Immortomouse<sup>+/-</sup>/Dmp1-GFP<sup>+/-</sup> long bones were performed to establish cell lines T1 and SW3, which are GFP-negative under maintenance conditions yet express GFP and produce different mineralized extracellular matrices under osteogenic culture conditions. T1 produces a honey-comb-patterned sheet of collagen fibrils that mineralize, increase in density by Day 7, and closely resemble MLO-A5 late osteoblast-like cells. In contrast, SW3 produces and mineralizes extracellular spherical structures similar to bone mineralization foci described for woven bone, UMR106, MC3T3-E1, and primary calvarial osteoblastic cells. SW3 cell lysates show steady GFP induction under osteogenic culture conditions from an undetectable baseline at day zero with peak expression at 14 days. Quantitation of alkaline phosphatase activity, Von Kossa and alizarin red staining, and expression of osteocyte marker E11/gp38 in SW3 cells show similar increases over time in culture. GFP and E11 co-localize with mineralized areas. We hypothesize that these cell lines represent different types of late osteoblasts with distinct mineralization processes that will be useful to study the osteoblast-to-osteocyte transition in conjunction with mineral deposition.