

Supplementary Information

S.1 Monitoring of MSCs Undergoing Osteogenic Differentiation

Progression of MSC differentiation into osteoblasts was monitored by observing mineralization and expression of MSC marker CD105 (Figure S1). Mineral deposition from mature osteoblasts is revealed by the Alizarin Red S stain as a red color (Figure S1a), and was observed as early as 7 days post-induction. Figure S1b shows a normalized increase in mineralization over time, with the largest increases on days 14 and 21, indicating a significant amount of differentiation occurring between 7 and 14 days, and then 14 and 21 days. Meanwhile, CD105 was strong in undifferentiated MSCs (Figure S1c), but steadily dropped off over 21 days of differentiation. An analysis of normalized fluorescent intensity (Figure S1d) showed that the largest decrease occurred between 0 and 7 days of osteogenic induction. The staining analysis in Figure S1 establishes an overall differentiation trend: MSCs show no mineralization and high expression of CD105 on day 0. By day 7, CD105 expression drops significantly, but cells show minor mineralization, suggesting the majority of cells are in an immature osteoblast progenitor stage. Significant mineralization occurs by day 14, and by day 21, when CD105 is absent, and osteoblasts are fully mineralized.

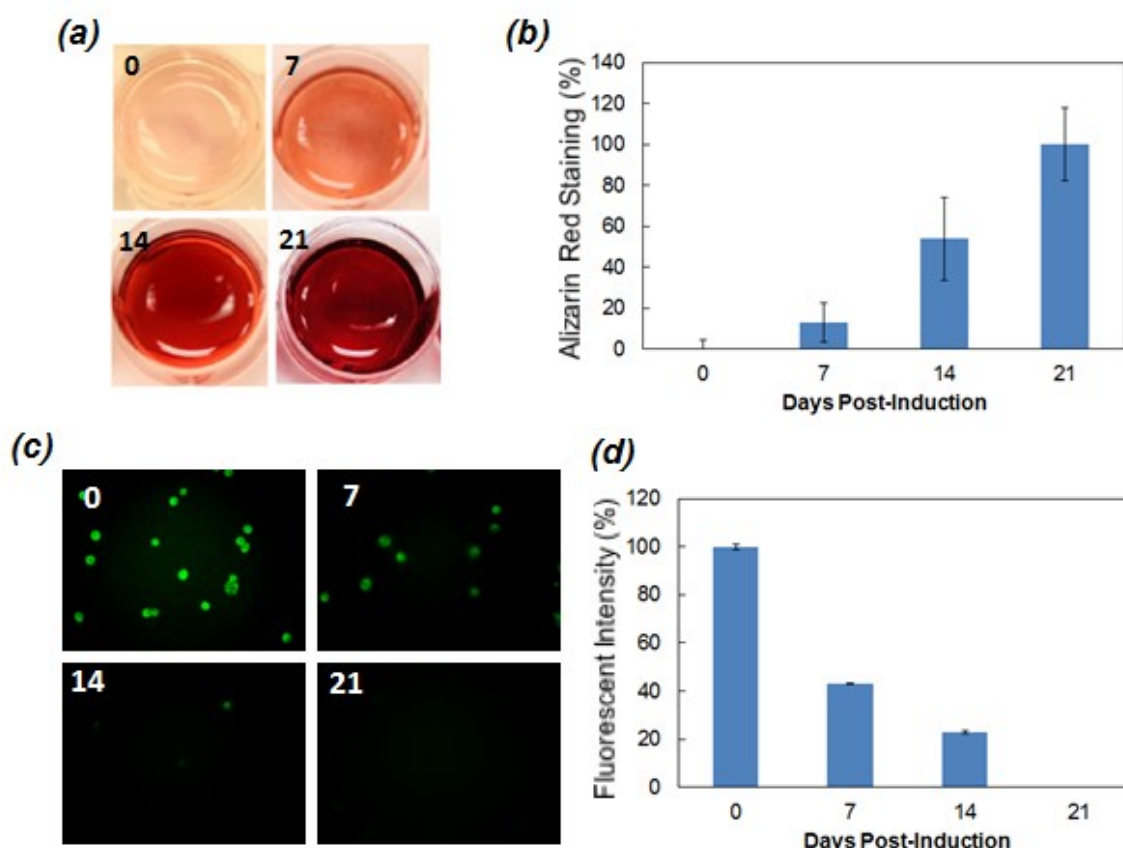


Figure S1. Monitoring of MSC differentiation into an osteoblast lineage. (a) Alizarin Red S staining shows progressive mineralization of differentiating cells; (b) Image analysis shows significant increases in mineralization at days 14 and 21, normalized to day 21; (c) CD105 expression is strong in MSCs, but decreases as the cells differentiate; (d) fluorescent intensity analysis of CD105, normalized to day 0, shows the largest decrease occurs by day 7, indicating a significant shift in population to a pre-osteoblast state.

