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Diamond nanocone array for improved osteoblastic differentiation

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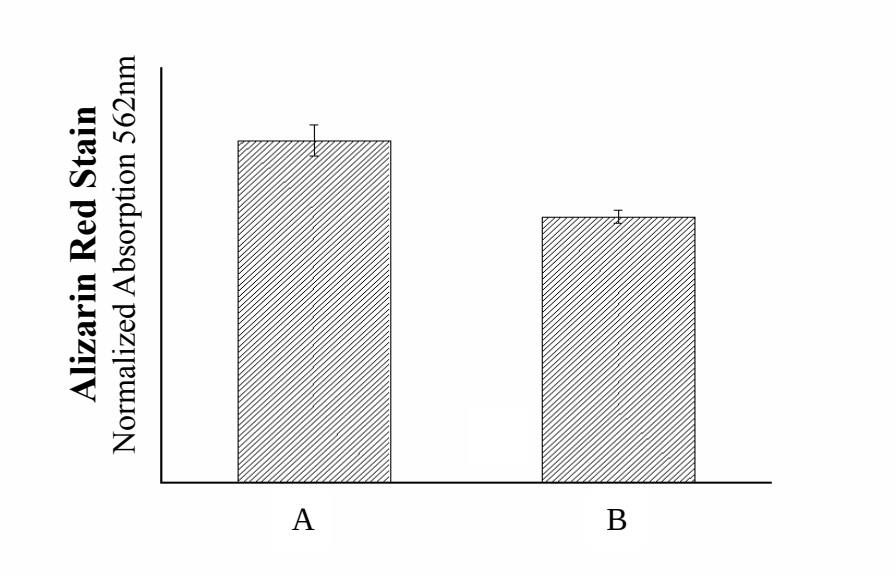

Supplementary Figure 1. Alizarin Red S staining assay for calcium deposition at 6 days after the addition of differentiation medium; A: Nanocone treated group; B: Smooth silicon substrate treated group.

Alizarin Red S staining was carried out to assess the degree of mineralization of osteoblastic MC3T3-E1 cells. The result clearly confirms the deposition of calcium by the osteoblastic cells. With nanocone treatment, the calcium deposition of the osteoblastic cells was about 30% higher than that produced by the cells with smooth silicon substrate treatment at only 6 days after the addition of differentiation medium ($p < 0.01$).

Experimental methods: MC-3T3 cells were treated with either nanocone or smooth silicon substrate as described in Section 2.3, and seeded to 12-well plate (3 wells for each group). The differentiation medium was replaced every 2 days. After 6 days post-culture, the cells were fixed with ethanol at room temperature for 1 hour and stained with 10% Alizarin Red (Acros) for 10 min. After washing 4 times with Millipore water (18.2 MΩ), the precipitate was solubilized in 10% cetylpyridinium chloride (J&K). Subsequently, the obtained solution was transferred to a 96-well microplate and the optical density was read at 562 nm by a microplate reader (Powerwave XS MQX200R).