Biomimetic Calcium Carbonate Concentric Microgrooves for Promoting MC3T3-E1 Cell Functions

Xiaohui Wu and Shanfeng Wang
Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996

Statement of Purpose: Biomimetic, self-assembled calcium carbonate ($CaCO_3$) concentric microgrooves with groove widths of 5.0 and 10 μm were fabricated through simply controlling incubation temperature for regulating mouse pre-osteoblastic MC3T3-E1 cell adhesion, spreading, alignment, nuclear deformation, proliferation, alkaline phosphatase activity, and calcium content.

Methods: The procedures for fabricating $CaCO_3$ substrates were based on a previous report.[1] MC3T3-E1 cells were seeded and cultured on all $CaCO_3$ substrates sterilized in excessive 70% alcohol solution, and characterized using the reported method.[2]

Results: With a similar depth of ~5.0 μm, two $CaCO_3$ groove widths of 10 and 5.0 μm were obtained at 37 °C and room temperature (~20 °C), respectively. Closely connected $CaCO_3$ concentric microgrooves were observed and the diameter of a ringed structure could extend to ca. 250 μm with ~10 successive concentric rings. Figure 1A shows MC3T3-E1 cell images and focal adhesions (FAs) in the cells after 1-day culture on both flat substrates and concentric microgrooves of $CaCO_3$. The F-actin-stained cytoskeletal images indicated that cells on flat $CaCO_3$ substrates were motile with very few stress fibers while the cells on the $CaCO_3$ microgrooves were well spread with more well-defined stress fibers, protrusions, and filopodia. The effect was more prominent when the groove width was smaller (5.0 μm). Cell protrusions on $CaCO_3$ microgrooves preferred to be aligned along the direction of the ridges. Vinculin-stained cells consistently had much more FAs on the microgrooves compared with those on the flat substrates. Again, the narrower microgrooves could induce more FAs. The FA density, i.e., the number of FAs per cell on $CaCO_3$ microgrooves, and the average FA area were larger than these on the flat substrates, and the values increased with decreasing the groove width from 10 to 5.0 μm (Figure 1B). FA circularity was calculated using the equation of $4\pi \times \text{area/perimeter}^2$, with a measure of 1 indicating a perfect circle. The lower circularity values on $CaCO_3$ microgrooves meant that FAs were more aligned. In addition, FAs on $CaCO_3$ microgrooves were mainly distributed on the ridges, besides on cell periphery. Similar to F-actin-stained cytoskeletal images, vinculin-stained images also showed that cells tended to grow along the groove direction, especially when the groove width was 5.0 μm. At longer time points, the cell densities on the 5.0-μm-wide grooves were significantly higher than those on the 10-μm-wide grooves, which were also significantly higher than those on the flat ones after day 2. At day 4, cell nuclear area was 130 ± 10 μm^2 on flat $CaCO_3$ substrates and it increased to 201 ± 18 μm^2 on 5.0-μm-wide $CaCO_3$ grooves. When the groove width was 10 μm, cell nuclei could be trapped inside the microgrooves and aligned along the groove direction after day 2 with an area as small as 115 ± 6 μm^2. After 14-day cell culture, the alkaline phosphatase activity and calcium content of the cells, two indicators of early-stage osteoblastic differentiation, on all substrates were semi-quantified by staining with fast blue RR and alizarin red S solutions, respectively. These two parameters were greatly enhanced on $CaCO_3$ microgrooves, especially on the 5.0-μm ones.

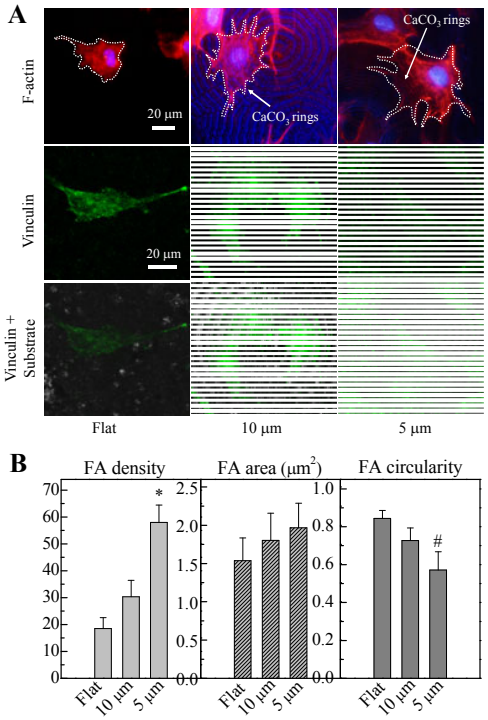

Figure 1. (A) Fluorescent images of MC3T3-E1 cells stained with rhodamine-phalloidin (RP, red) and 4',6-diamidino-2-phenylindole (DAPI, blue) (top row), vinculin-stained images (middle row), and merged images with the $CaCO_3$ background (bottom row) at day 1 post-seeding. The dotted lines mark the cell contour. Scale bar of 20 μm is applicable for all. (B) Density, area, and circularity of FAs measured from vinculin-stained cell images in (A). *: $p < 0.05$, #: $p < 0.01$: relative to the flat.

Conclusions: Microgrooves in $CaCO_3$ substrates could dramatically enhance MC3T3-E1 cell adhesion, spreading, proliferation, and differentiation, especially on the narrower ones. Cell nuclear distribution and distortion (either being aligned or expanded) were also quantified to show that more aligned cell nuclei were trapped in 10-μm-wide $CaCO_3$ grooves because of their comparable dimensions while cell nuclei with a larger area could span over several 5-μm-wide ones. This study showed the great promise of using biomimetic $CaCO_3$ concentric microgrooved topography in bone tissue engineering applications.

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References: 1. Sakamoto T. *Cryst. Growth Des.* **2009**, *9*, 622. 2. Wu X. *ACS Appl. Mater. Interf.* **2012**, *4*, 4966.