Role of Citric Acid on Apatite Nucleation and Osteogenic Differentiation of Human Mesenchymal Stem Cells on Aligned Nanofibers

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Statement of Purpose: It has been shown that the spacing between carboxylate groups matches those between calcium ions in the growing apatite nanocrystals. Considering that carboxylate-rich organic acids account for 5.5% of the organic matter in bone, they should play a key role in controlling crystal growth and size. The objective of this work was to investigate the effect of organic acids, more specifically citric acid in combination with ascorbic acid, on apatite nucleation and osteogenic differentiation of human mesenchymal stem cells (hMSCs) on glutamic acid (Glu)-functionalized aligned nanofiber microsheets. Organic acids included hydroxyl citric acid (HCA), citric acid (CA), tartaric acid (TART), maleic acid (MA), and salicylic acid (SalA), and ascorbic acid (AsA).

Methods: Cysteine-terminated EEGGC (GLU) peptide was synthesized in the solid phase. The peptide was conjugated to low molecular weight acrylated poly(L-lactide) (PLA) via Michael addition to synthesize the PLA-Glu conjugate. Aligned PLA nanofibers sheets were electrospun from a 10 wt% solution of high molecular weight PLA and PLA-Glu with 1.0 mL/h injection rate, 20 kV electrical potential, and 7.5 cm needle-to-collector distance and the fibers were collected on a rotating wheel at 1200 rpm. The nanofiber sheets were incubated in a modified solution of 10x concentrated simulated body fluid (SBF) supplemented with one of the organic acids. The amount of CaP nucleation on the nanofibers was characterized using a QuantiChrom calcium assay. The elemental composition of the samples was analyzed using an Energy Dispersive X-ray Spectrometer (EDS) connected to FESEM at an accelerating voltage of 15 kV. Next, hMSCs were seeded on the functionalized nanofibers and cultivated in osteogenic medium supplemented with one of the organic acids (HCA, CA, TART, MA, SalA). At each time point, the cell-seeded fibers were evaluated for cellularity, alkaline phosphatase (ALP) activity, the extent of mineralization, and bone nodule formation.

Results: Addition of organic acids to SBF significantly increased CaP nucleation on the fiber microsheets and the extent of CaP nucleation depended on the number of carboxylic acid and hydroxyl groups in the organic acid (Figure 1). HCA-supplemented group had the highest CaP content at 240±10% followed by TART and CA with 225±8% and 225±10%, respectively. CaP nanoparticles nucleated on the fiber surface in TART-supplemented group had Ca/P ratio (1.68) and crystallinity (39%) closet to that of natural bone. Microsheets in HCA-supplemented group had the highest compressive modulus of 2400±140 MPa followed by TART and CA with 2350±120 and 2250±140 MPa, respectively. Addition of HCA, TART, MA, and SalA to osteogenic medium inhibited osteogenic differentiation of hMSCs seeded on the nanofiber microsheets.

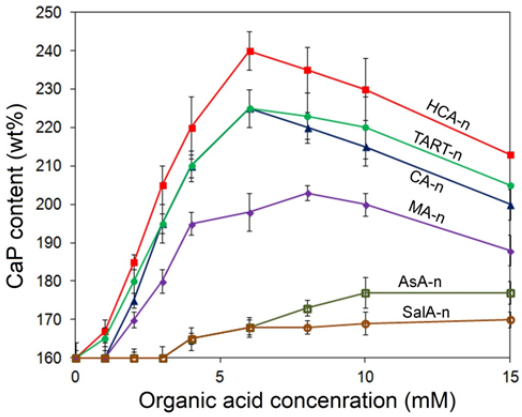

Figure 1. Effect of supplementing SBF with organic acids on nucleation of calcium phosphate crystals on the surface of aligned PLA-Glu nanofibers.

Osteogenic differentiation of hMSCs was enhanced only with the addition of CA to the osteogenic medium (Figure 2A). CA-supplemented group also stimulated the formation of bone nodules on the microsheets (Figure 2B) compared to the control (Figure 2C).

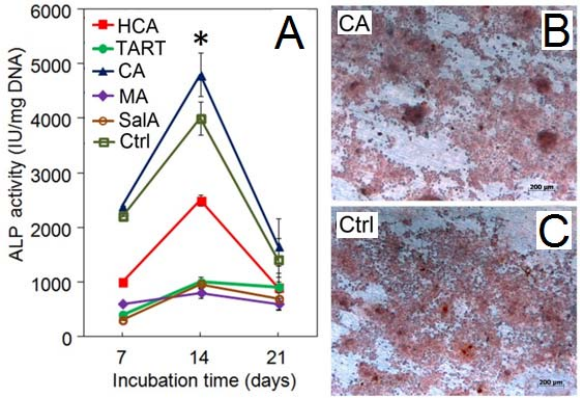

Figure 2. (A) Effect of supplementing the osteogenic culture medium with organic acids on ALP activity of hMSCs on aligned PLA-Glu nanofibers; Alizarin red staining of the hMSCs cultivated in osteogenic medium (C) and osteogenic medium supplemented with CA (B).

Conclusions: Results demonstrate that ascorbic acid in the osteogenic medium and the added citric acid synergistically enhanced osteogenic differentiation of hMSCs, mineralization, and nodule formation.

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