

Aseptic loosening and bacterial infection are the two leading causes of implants' failure. The recently developed additively manufacturing techniques have enabled fabrication of porous biomaterials to mimic native bone characteristics. In this research, we aimed to biofunctionalize the surface of additively manufactured Ti-6Al-4V implants using plasma electrolytic oxidation (PEO), which is capable of embedding bioactive elements in the oxide matrix. We also applied a subsequent hydrothermal treatment to synthesize hydroxyapatite nanocrystals throughout the oxide layer. The formation of HA nanocrystals was found to be interpreted according to the supersaturation of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  during the hydrothermal process. At the beginning, the high local supersaturation resulted in a homogenous nucleation of spindle-like nanocrystals all over the surface. By continuing the process, depletion of reactant ions in the outermost surface layer lead to a remarkable decrease in supersaturation values and therefore, high aspect-ratio nanorods and hexagonal nanopillars were generated. Owing to the not ideal structure and dimensions, however, the implants' surfaces coated with HA nanopillars had a negative impact on proliferation and osteogenic differentiation of pre-osteoblastic MC3T3-E1 cells. By contrast, the unique hierarchical structure of micro-porous PEO layer ( $< 3 \mu\text{m}$ ) and spindle-like nanocrystals ( $< 100 \text{ nm}$ ) on the surface of macro-porous additively manufactured implants provided a favorable anchorage substrate for cytoplasmic extensions to easily attach and move on the surface.