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Biomineralization of Cellulose Nanocrystal Scaffolds for Bone Tissue Applications F. A. Abushahba^{1, 2}, J. Borges-Vilches³, S. Mohamed⁴, T. J. Heino⁴, E. Kontturi³, P. Vallittu¹ ¹Department of Biomaterials Science and Turku Clinical Biomaterial Center-TCBC, Institute of Dentistry, University of Turku, Turku, Finland, ²Department of Prosthetic Dentistry and Stomatognathic Physiology, Institute of Dentistry, University of Turku, Turku, Finland, ³Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, Aalto, Finland, ⁴Institute of Biomedicine, Faculty of Medicine, University of Turku, Turku, Finland

Objectives This study aimed to biomineralize cellulose nanocrystals (CNC) scaffolds and evaluate their biocompatibility with osteoblast cells as a potential bone graft substitute. **Methods** CNC-reinforced gelatin scaffolds were produced using a casting solution method followed by freeze-drying. An ether-derived compound, 1,4-butanediol diglycidyl ether, was used for crosslinking to promote the formation of covalent bonds between gelatin and CNC. The CNC scaffolds were then cut into \emptyset 12 mm discs (n = 21) of 3 mm thickness. Before mineralization in simulated body fluid (SBF), the CNC scaffolds were alternately soaked in calcium chloride and sodium phosphate solutions for 15 min each. The samples were then mineralized in 1.5-fold 20 ml SBF at 37° C for 24, 48, 72, and 96 h. The Calcium (Ca²⁺) and Phosphate (PO₄³⁻) ion concentrations in the SBF were measured by inductively coupled plasma-optical emission spectrometer (ICP-OES) at all time points. The mineralized CNC scaffolds were also characterized by scanning electron microscope (SEM) and energy dispersive x-ray spectroscopy (EDS) analysis. The MC3T3-E1 osteoblast cell proliferation was assessed on samples mineralized for 96 h after 24 h of culture.

Results ICP-OES analysis showed a significant (p < .001) decrease in the Ca²⁺ and PO₄³⁻ concentration in the mineralization solution at all time points compared to fresh SBF. Consequently, this resulted in a notable increase in the weight of the CNC scaffolds by 50% at 48 h and later time points. Also, SEM-EDS analysis of the surface and cross-section of the CNC scaffolds showed the presence of Calcium-Phosphate compositions on the surface and inside the scaffolds. Compared to the control, more than two-fold osteoblast cell proliferation was observed on the mineralized CNC scaffolds.

Conclusions Biomineralized CNC scaffolds are not only biocompatible, but they can also enhance the proliferation of osteoblast cells.