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Functionalization of Titanium-based surfaces with 3D polymeric microstructures fabricated by Laser Direct Writing via Two Photon Polymerization

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Titanium-based implants are frequently used in medical practice for bone-associated injuries and disorders. However, the osteointegration of Titanium is limited by its lack of bioactivity. To overcome this drawback, in this study we functionalized titanium-based surfaces with 3D polymeric microstructures fabricated by laser direct writing via two-photon photopolymerization (LDW via TPP), which improved the osteogenic response of osteoblast-like cells . The microstructures consisted of 64 vertical microtubes, arranged on 5 layers of different heights (from 10 to 170 μm), having a porosity ranging from 94% to 99% (consistent with the upper limit porosity of natural bone). The microstructures were made from a biocompatible photopolymerizable photopolymer (Ip-Dip). MG-63 osteoblast like cells were seeded on the 3D polymeric microstructures and analyzed at 1, 2, 7 and 14 days. For comparison, we also studied the behaviour of MG-63 cells seeded on non-functionalized Titanium-based surfaces. Scanning electron microscopy (SEM) investigations showed that the 3D polymeric microstructures promoted the three-dimensional organisation of the seeded cells, which is characteristic of bone tissue, whereas the cells on non-functionalized titanium surfaces were arranged only in 2D. In addition, SEM analysis evidenced earlier signs of collagen fibrils synthesis (namely after only 7 days) in the cells from Titaniun surfaces functionalized with 3D polymeric microstructure, as compared to slower collagen synthesis (after 14 days) for cells on non-functionalized Titanium. Moreover, Energy Dispersive X-ray Spectroscopy (EDX) indicated incipient signs of mineralization, as shown by early-stage presence of calcium and phosphorus within seeded cells, that for Titanium-based surfaces functionalized with 3D polymeric microstructureshat started from two weeks of cells cultures. Finally, we determined the traction forces exerted by the osteoblast cells on the 3D polymeric microstructures, given that these forces are known to strongly influence the cellular behaviour. To this end, we performed finite element analysis (FEA) simulations in the FEBio Studio environment. First, we simulated, as close as possible ,the in vitro experiment, using the mechanical properties of Titanium and of Ip-Dip photopolymer. The displacements of microtubes's tips during in vitro experiments were measured by SEM (namely, 17.6 ± 0.2 μm displacement for 130 μm microtubes's height). The simulation indicated that the traction force required to induce such a displacament was of approximately 600 μN, which is approximately three orders of magnitude higher than the traction forces reported for osteoblast cells in vitro. We believed that this difference arrised from the fact that the mechanical properties of Ip-Dip photopolymer were much different than the values of materials encountered in vivo. As such, to validate our approach, the simulation was repeated using the mechanical parameters specific to bone extracellular matrix, in which case the estimated force was of 5.5 nN –a value consistent with the traction forces reported in the literature. In all, these results highlight the promising premises of the proposed approach of functionalizing titanium-based surfaces with 3D polymeric microstructures fabricated by LDW via TPP , to support the osteointegration process of bone implants and, consequently, to improve the patient's quality of life.

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