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BONE SCAFFOLDS IN SIMULATED MICROGRAVITY: AN EXPERIMENTAL APPROACH TO  
ASSESS CELL RESPONSE TO A BIOMIMETIC MICROENVIRONMENT

**Abstract**

Microgravity is a very harsh condition for biological systems, eliciting a negative outcome that needs to be critically assessed. Data collection, critical analysis and tailored countermeasures can effectively concur to plan safe long-term manned space missions for different gravity environments like the International Space Station (ISS), Moon, and Mars, being the current and the next scenarios of Space exploration. In this respect, tissue engineering can support the research in the field with the aim to collect more detailed results if using biomimetic scaffolds. Focussing on trabecular bone tissue, which is exposed to a significant demineralization induced by weightlessness, this study presents the cell response to 3D printed scaffolds mimicking the microarchitecture of healthy and osteoporotic bones in simulated microgravity. Scaffold design is a key topic as the instructive role that it is expected to exert on cells by means of an appropriate three-dimensional microarchitecture can correctly support biological processes and lead to a more realistic output. Furthermore, composite replicas including  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) to better reproduce bone composition were also assessed. Both physiologic and pathologic fabricated trabecular structures (4x4x4 mm<sup>3</sup>) were tested culturing the osteosarcoma cell line (SAOS2), as a bone model, in a rotary cell culture system (RCCS) at 40-45 rpm. The rotational speed of the RCCS was adjusted to compensate for cell sedimentation rates and to allow the correct positioning of the scaffolds within the bioreactor, which depends on their size, weight, and density. Results are evaluated in terms of cell growth rate, cell cycle and metabolism analyses, alkaline phosphatase assay, and mRNA expression of osteoblast differentiation markers (AP, OPN, OCL, RUNX-2). The proposed experimental approach can pave the way to the development of a refined protocol to investigate bone cell response to microgravity conditions. Biological data show that the scaffolds may support the osteogenic differentiation, being related to the bone-like architecture, underlining their biomimetic and bioactive potential.