In vitro cytocompatibility evaluation of poly(DL-lactic acid) scaffolds loaded with minocycline and voriconazole addressing osteomyelitis

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Osteomyelitis or bone infection is an acute or chronic inflammatory process involving the bone and its structures, secondary to infection with pyogenic organisms, as bacteria and fungi. Considering the associated high patient economic burden, morbidity and mortality, it is essential to develop novel strategies for osteomyelitis management. Porous scaffolds based in biomaterials may locally deliver high concentrations of antibiotics, an effective strategy in eradicating bone infection. When incorporating bioactive bioglasses and bioresorbable polymers as poly(DL-lactic acid) (PDLLA), these structures exhibit biosafety, biodegradability and the expected global structure to promote cell expansion and cell differentiation, being critical to consider and evaluate their biocompatibility compliance. As the encapsulation of more than one active pharmaceutical ingredient is an attractive approach, the present study focuses in the cytocompatibility evaluation of an innovative system based in the dual delivery of two antimicrobials, an antibiotic that enhances bone formation, minocycline (MH) and an antifungal agent with a broad spectrum of activity, voriconazole (VCZ), aiming bone infection therapeutics.

Scaffolds were prepared by solvent casting/particulate leaching techniques and functionalized with bioglass [1]. The scaffolds produced were adsorbed with 0.5 or 0.1 mg/mL of minocycline and also with 0.1 mg/mL of voriconazole. To test the bio-functionality and the biological safety of scaffolds, *in vitro* cell assays were achieved employing the MG-63 cell line (ATCC® CRL-1427™ human osteoblast cell line). The AlamarBlue® assay was used to measure cell proliferation in the scaffold [2]. As osteoblast differentiation markers, the following were determined: alkaline phosphatase activity and mineralization using Alizarin red assay, an indicator of *in vitro* bone formation [3].

All scaffolds sustained the proliferation of metabolically active cells, nonetheless, scaffolds adsorbed with the highest concentration of MH (0.5 mg/mL) presented a significant ($p<0.05$) cytotoxic effect. Matrix maturation assays supported early osteoblasts differentiation and the osteoinductive role of minocycline described in the literature was also highlighted. Matrix mineralization analysis showed the highest value associated with scaffolds with both antimicrobials adsorbed.

Once the described scaffolds enhanced osteoblasts differentiation, matrix mineralization and evidenced no cytotoxic effects, they come to light as an auspicious alternative for local antimicrobial therapy addressing osteomyelitis prevention and therapeutics.

Keywords: Cytocompatibility; osteomyelitis; co-delivery; osteoblasts cellular differentiation; scaffolds.

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