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In vitro Antitumor Efficiency Screening of Methotrexate - loaded Hydroxyapatite/PLGA Nanostructured Coatings †

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Abstract: Globally, osteosarcoma accounts for 20% of the total cases of primary malignant bone tumors, and unfortunately, it affects mainly children and young adults. Osteosarcoma is characterized by a high degree of malignancy, strong invasiveness, rapid disease progression, and an extremely high mortality rate. The primary treatment for osteosarcoma management is based on surgery and chemotherapy, with high-dose methotrexate (HDMTX) being the backbone of the chemotherapy regiment. However, despite its efficacy, HDMTX administration exhibits increased toxicity on normal tissues which triggers severe adverse reactions such as liver and kidney function damage, bone marrow suppression, neurotoxicity, and gastrointestinal reactions. In this view, delivery of MTX through implantable biomaterials could address these limitations imposed by conventional therapy by modulating MTX release and ensuring that the drug is mainly delivered to osteosarcoma tumor cells. Therefore, our study aimed to develop an implantable biomaterial based on hydroxyapatite-PLGA nanostructures loaded with MTX deposited through a laser processing method, namely MAPLE (Matrix Assisted Pulsed Laser Evaporation) on titanium disc surfaces. After nanostructured coatings synthesis and characterization, samples were sterilized and used for *in vitro* biological investigations on two cellular models: osteosarcoma human tumor cells SaSo2 and human preosteoblasts hFOB 1.19. 24h and 72h post-cell seeding, the experimental samples were subjected to various assays to reveal biomaterials cytotoxicity, impact on cell viability and proliferation, and cell typical morphology by spectrophotometry (MTT assay) and fluorescence microscopy (Live/Dead and actin filaments staining by FITC-phalloidin). Our results revealed that pristine materials exhibit excellent *in vitro* biocompatibility, with no cytotoxic effects being noticed in contact with the two cell lines. In contrast, when loaded with MTX, the materials determine a significant decrease of cell viability and proliferation potential of SaSo-2 osteosarcoma tumor cells, together with severe alterations of cell typical morphology and actin expression. With respect to hFOB 1.19 cells cultured on MTX-loaded samples, cell viability and proliferation potential were slightly reduced, and no notable alterations of the cytoskeleton were observed, showing that the cytotoxic effect of MTX-loaded samples is significantly higher on tumor cells as compared with normal cells. This capacity of the novel MTX-loaded material to exhibit cytotoxic effects selectively on tumor cells holds great promise in using this approach for osteosarcoma management, but further *in vivo* studies are required to validate this novel therapeutic strategy.

Keywords: osteosarcoma; MAPLE; cancer management.

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Conflicts of Interest

The authors declare no conflict of interest.