A 3D *in vitro* cell culture model based on perfused bone-like scaffolds for healthy and pathological bone research

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INTRODUCTION: Comprehensive research, particularly in evaluating drug efficacy**,** still heavily relies on the results obtained by the utilization of cell monolayers and animals. However, the inherent limitations of these models such as their physiological disparities from humans pose significant obstacles to acquiring reliable results thus impeding further scientific progression. To address this challenge, 3D *in vitro* cell culture models emerged as physiologically relevant models having the potential to enhance research and drug discovery. Our study aimed to develop a 3D *in vitro* cell culture model based on bone-like scaffolds in conjunction with a perfusion bioreactor ("3D Perfuse", Innovation Center FTM, Belgrade, Serbia) for studying both physiological and pathological (i.e. tumors) bone conditions.

EXPERIMENTAL: Bone-like scaffolds were obtained by cross-linking the mixture of Na-alginate solution (2 wt.%) and hydroxyapatite (2 wt.%) with calcium ions followed by slow freezing and lyophilization. Scaffold porosity and pore sizes were determined by using optical microscopy. To model osteosarcoma tumor**,** scaffolds were seeded with murine K7M2-wt osteosarcoma cells**,** whereas for mimicking bone physiological conditions either human bone marrow-derived mesenchymal stem cell line (hBMSCs) or primary mesenchymal stem cells were used. Each cell type was cultivated for 7 days in a perfusion bioreactor with medium flow rate of 0.27 cm^3/min, corresponding to the medium superficial velocity of 40 μm/s. Static cell cultures served as controls. Cell behavior was assessed by cell metabolic activity assays (MTT or resazurin), histological and immunocytochemical analysis, phalloidin/DAPI staining, and gene expression analysis (qPCR). Shear stresses were calculated from histological sections using a cylindrical pore model.

RESULTS AND DISCUSSION: Obtained scaffolds had an initial porosity of 60 % and contained a variety of pore sizes with a predominant presence of macropores, mimicking in that manner trabecular bone structure. All cell types adhered to the scaffolds indicated by cell seeding efficiency exceeding 80 %. In perfusion culture, osteosarcoma cells exhibited characteristics corresponding to *in vivo* tumor cell behavior: high cell metabolic activity, spontaneous assembly into compact spheroid-like structures, secretion of extracellular matrix and expression of pluripotency-associated genes. Furthermore, immunocytochemical staining revealed an increased presence of α-tubulin as compared to the control. Regarding the healthy bone model, both types of mesenchymal stem cells retained their intrinsic cellular shape and self-organized into aligned structures which was strongly induced by perfusion conditions. The overall positive influence of perfusion conditions could be attributed not only to improved mass transport but also to adequate values of hydrodynamic shear stresses calculated to be up to 5 mPa.

CONCLUSIONS: Our 3D *in vitro* model can support cultures of different bone cell types and shows potential for further adjustment and utilization in the fields of tumor and tissue engineering.

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