Bioengineering for creating biomimetic microenvironments: bioreactors and biomaterials

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INTRODUCTION: Millions of patients are still awaiting new therapies as the traditional models rely on monolayer cell (2D) cultures and *in vivo* studies, which have numerous limitations resulting in misleading conclusions. Consequently, there is a burning need for the development of alternative 3D models able to accurately mimic the complexity of human diseases. This research aim is to create microenvironments based on biomimetic bioreactors and alginate hydrogels as cell carriers for reliable disease research and drug screening.

EXPERIMENTAL: Alginate cell carriers in forms of microfibers and microbeads with immobilized different cells were obtained by extrusion techniques [1], while macroporous cell carriers for imitating bone tissue based on alginate or gellan gum and bioactive inorganic particles (hydroxyapatite, ß-tricalcium phosphate, bioactive glass) were prepared by a simple controlled gelation and freeze-drying method [2] followed by manual seeding of cells onto the partially rehydrated scaffolds. The obtained carriers with cells were cultivated in perfusion bioreactors ("3D Perfuse", Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia) under continuous medium flow (superficial velocity: 15-100 µm s^{-1}) for up to 7 days. To evaluate these cell carriers for drug screening, microfibers with different cancer cells were treated with cisplatin or doxorubicin, while 2D cultures served as control. The cells were assessed regarding the metabolic activity (viability) by MTT, morphology, and distribution within carriers by scanning electron microscopy and histology (H&E stain).

RESULTS AND DISCUSSION: Cell immobilization in alginate microbeads (diameter: ~300 µm) and microfibers (diameter in the range 300-500 µm) resulted in uniform distribution, while the macroporous scaffolds with open and connected pores (porosity: ~60 %) provided cell adherence as individual cells and in aggregates (seeding efficiency: above 80 %).The majority of cells stayed viable and metabolically active, while retrieved cells from alginate carriers retained their morphology, viability, and ability to proliferate under 2D conditions. Alginate carriers with cervical carcinoma, glioblastoma, and osteosarcoma cells were further cultivated in perfusion bioreactors. After cultivation under biomimetic conditions the cells retained viability and proliferative capacity, spontaneously formed spheroid-like structures, and exhibited higher metabolic activity as compared to static controls. The obtained results imply the positive effects of medium flow on cells due to providing efficient mass transport and controlled levels of hydrodynamic shear stresses. Evaluation of these models for drug screening has shown that the immobilized different cancer cells exhibited up to 10-fold higher half-maximal inhibitory concentration than the cells in 2D cultures.

CONCLUSIONS: The overall results have shown potentials of the applied approach based on 3D models comprising biomimetic bioreactors and alginate-based scaffolds for disease research and drug screening.

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