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Short communication

Tumor mimetic platform for clinically relevant novel drug screening

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Introduction

Pharmaceutical companies spend ~$2.6 B and time of 10-15 years on the research and development of a new drug candidate (Avorn, 2015). Unfortunately, less than 10% of these candidates are approved by FDA (Wong et al., 2019), due to the weaknesses of preclinical drug evaluation methods based on conventional monolayer cell cultures and different animal models, which do not adequately imitate the complexity of human diseases such as cancer. Therefore, pharmaceutical companies are looking for reliable, relevant, reproducible and inexpensive preclinical models capable to faithfully replicate *in vivo* microenvironment so to improve the drug development process.

Tissue engineering principles are recognized as a promising strategy to create an artificial *in vivo*-like environment based on biomaterials as cell carriers that imitate native extracellular matrices (ECMs) and biomimetic bioreactors providing physiologically-relevant conditions. The aim of this work was to create and validate a novel, simple, relevant, and robust 3D platform for anticancer drug screening, based on alginate hydrogels as cell carriers and a biomimetic perfusion bioreactor that imitates the environment of highly vascularized tissues.

Materials and methods

Alginate carriers in forms of microfibers and microbeads with immobilized different human and animal cancer cell lines (embryonal carcinoma NT2/D1, cervical carcinoma SiHa, lung cancer NCI-H460, U87 glioblastoma, murine K7M2-wt osteosarcoma and rat C6 glioblastoma) were produced by extrusion techniques, as described earlier (Stojkovska et al., 2020; Radonjic et al., 2022). In brief, alginate microfibers were produced by simple, manual extrusion of Na-alginate solutions (1.3 - 2.8% w/w) with cells (1 - 4 million cells/ml), into a gelling bath containing 0.18 M Ca or 0.045 - 0.18 M Ba ions, while microbeads with immobilized cells were produced by electrostatic droplet generation using 4.3 kV electrostatic potential, 2.5 cm electrode distance, blunt edge needle, 25.2 ml/h flowrate and a gelling solution containing 0.18 M Ca ions. The obtained microbeads and microfibers were cultivated up to 28 days under static conditions, and in a perfusion bioreactor ("3D Perfuse", Innovation Center FTM, Belgrade, Serbia) at continuous medium flow (superficial velocities: 15 – 100 μm/s) in short term studies up to 5 days. In order to evaluate this platform for anticancer drug testing microfibers with NCI-H460 and K7M2-wt cells were treated after 24 h of immobilization with cisplatin (0.5 - 25 μM) and doxorubicin (0.25 - 20 μM), respectively, while cells in 2D served as control. In

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addition, microfibers with U87 glioblastoma cells were treated with 100 µM temozolomide, which started on day 7 and lasted for 3 consecutive days with a recovery period of 18 days in order to validate platform for assessment of anticancer drug effects in a clinically relevant schedule.

The alginate carriers were assessed regarding the cell metabolic activity (viability) by MTT and Live/dead assays, as well as regarding morphology and distribution within carriers by histological (H&E stain) and immunohistological (α-tubulin) analyses. Expression of resistant related genes was assessed by quantitative real-time PCR (qRT-PCR).

Results and discussion

Alginate microfibers (diameter in the range 300-500 µm, Fig. 1a) with uniformly immobilized cells (4 million cells/ml) were produced by manual extrusion of cell/alginate suspension, while spherical and uniform microbeads (~300 µm in diameter, Fig. 1b) with immobilized cells (1 million cells/ml) were produced by electrostatic extrusion. The majority of immobilized cells remained viable and showed metabolic activity, while retrieved cells from alginate carriers retained their morphology and viability, as well as ability to adhere to the culture plastic and proliferate under 2D conditions.

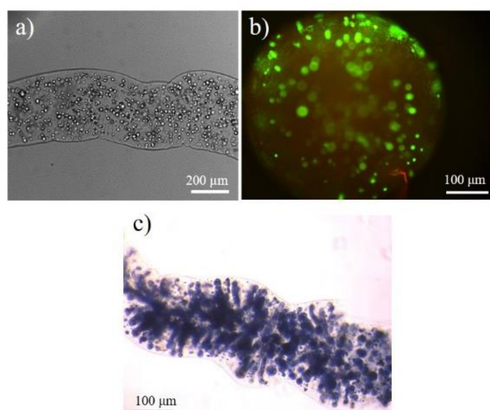

Fig. 1. Alginate carriers with immobilized cells: a) optical micrograph of the initial alginate microfiber with U251 cells, b) Live/Dead image of NT2/D1 cells in a microbead after immobilization, c) optical micrograph of MTT-stained viable SiHa cells within microfibers cultured for 10 days under static conditions

Over 4 weeks of static cultivation cells in alginate carriers spontaneously formed aggregates oriented to the carrier's periphery towards the source of oxygen and nutrients, due to mass transport limitations (Fig. 1c).

In order to provide efficient mass transport and adequate shear stresses, microfibers with rat C6 glioblastoma cells were cultivated in perfusion bioreactors under continuous medium flow at continuous medium flow (superficial velocities of 100 µm/s). After 5 days of cultivation under biomimetic conditions the cells retained viability, proliferative capacity and exhibited higher metabolic activity as compared to static controls (Radonjic et al., 2022).

Evaluation of the platform for high-throughput anticancer drug screening has shown that the immobilized NCI-H460 and K7M2-wt cells exhibited up to 10-fold higher half-maximal inhibitory concentration as compared to 2D cultures. On the other hand, evaluation of the platform for assessment of drug effects on cells treated in a clinically relevant schedule has shown anti-proliferative effects of drug in both 2D and 3D cultures, being higher in the former case. These results are due to the significantly increased drug resistance-related gene expression in 3D immobilized U87 cells as compared to 2D cultures (Dragoj et al, 2021).

Conclusion

The overall results of the present study demonstrated benefits of the tumor mimetic platform based on alginate hydrogels as cell carriers in conjunction with the perfusion bioreactor for relevant and reliable high-throughput anticancer drug screening, as well as for assessment of drug effects applied in a clinically relevant schedule.

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