Adaptable alginate-based microfibers for 3D *in vitro* cultures of cancer cells: an anticancer drug testing model

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INTRODUCTION: The slow advance in anticancer drug development can be attributed to the limitations of conventional models, predominantly monolayer cell (2D) cultures and animal models, which inadequately recapitulate the complex nature of human malignant tumors. Three-dimensional (3D) *in vitro* models are invaluable tools in drug screening; however, creating a universal model for all cancer types poses challenges due to the diverse nature of cancers. The aim of this work was to develop a single, versatile model using alginate microfibers to accommodate cultivation of various cancer cells.

EXPERIMENTAL: Two cancer cell types were used: osteosarcoma (human HOS and U2OS, and murine K7M2-wt cell lines) and non-small cell lung carcinoma (NCI-H460 human cell line). Cells were suspended (4×10^6 cells cm^{-3}) in alginate solution (2 or 2.8 wt.%) or in a solution containing 2 wt. % alginate and 2 wt. % commercial hydroxyapatite (HAP) powder. To obtain microfibers, the suspensions were manually extruded through a 25 or 26-gauge needle into the gelling bath containing 0.18 M Ca^{2+} or 0.045 M Ba^{2+}. The obtained microfibers were washed and transferred into culture flasks and then cultured up to 21 days. The 3D cultures were validated in anticancer drug testing: 3 cm of microfibers per well in a 96-well plate were treated with 0.25-20 µM doxorubicin (K7M2-wt) or 0.5-50 µM cisplatin (NCI-H460). Treated cells in monolayer served as a control. The viability and distribution of the cells were examined using live/dead assay and histology (H&E staining). The half-maximal inhibitory concentration (IC_{50}) was determined by the MTT assay.

RESULTS AND DISCUSSION: The obtained results of osteosarcoma cells immobilized in Ca-alginate microfibers with and without HAP, and lung cancer cells immobilized in Ba-alginate microfibers have shown that the microfibers supported cell viability, metabolic activity, and formation of cellular aggregates [1]. The results of anticancer drug testing have shown that IC_{50} values for K7M2-wt cells immobilized in alginate microfibers with and without HAP, as well as for the 3D cultures of NCI-H460 cells were up to ~10-fold higher than the IC_{50} values of 2D cultures. These results align with the observed higher resistance to anticancer drugs in patients compared to traditional preclinical models.

CONCLUSIONS: These findings demonstrate the potentials of the developed 3D model for more reliable anticancer drug screening and enhancement of the preclinical platforms for drug discovery.

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