Alginate-based 3D Cell Phantom for Radiobiological Experiments

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Background and Objectives

Cell culture has been one of the most common tools for the discoveries and testing of different cancer treatments since the 1900s. Traditionally, cell monolayers are used for this purpose. Despite the ease of use, there are, however, several **drawbacks of using 2D cultures**:

- Equal exposure of cells get to nutrients, oxygen, or toxins, while in living organisms cells have a gradient exposure to these factors within 3D structures.
- Only cell-cell interactions, while in living organisms cells also interact with their environment => different response to medications or radiation exposure compared to living tissues.

3D cell cultures are considered to mimic the real tissues better than a monolayer. Numerous studies report different protocols for gel-based 3D cell cultures. However, most of them are either based on expensive materials, or not applicable for selected types of radiobiological experiments.

Goal: to develop a relatively inexpensive 3D cell culture phantom out of stable, rigid gels, but without sacrificing the cell proliferation and viability. These gels should be sufficient for the experiments, studying cellular effects after the irradiation with ion beams. In this work, a protocol for cell culture in gels, consisting of alginate, type I collagen, and silk fibroin, was established.

Methods

Gel: alginate (AG) 0.25%, collagen type I (COLI) 0.1875%, silk fibroin (SF) 0.25%, and cells (1.75 x 10^6 cells/ml).

Cell lines: U2OS (bone osteosarcoma) and A549 (lung carcinoma).

Preparation:

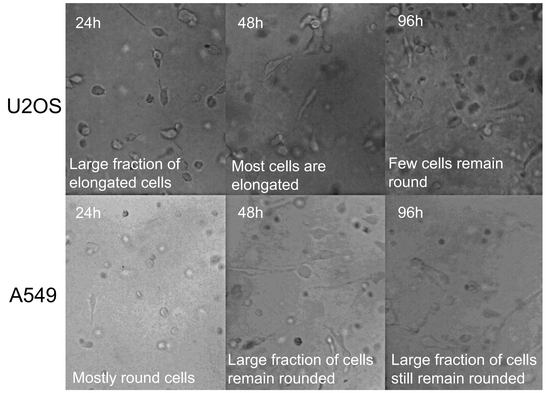
- Gelatine molds containing 100 mM CaCl2 for alginate ionic cross-linking were prepared inside a 12-well plate.
- COLI was neutralized using 1N NaOH and 10x PBS and mixed on ice with AG, SF, cell suspension, and culture medium. The mixture was poured on gelatine molds and incubated for 40 minutes until solid.
- After the incubation, gels were washed in 10mM CaCl2 solution, PBS with Ca ions, and twice with medium.
- Medium exchange: every 2-3 days.

Cell growth analysis: gels were dissolved using PBS with 0.046% EDTA and cell numbers and viability were estimated using trypan blue dye and Bio-Rad TC20 Automated Cell Counter.

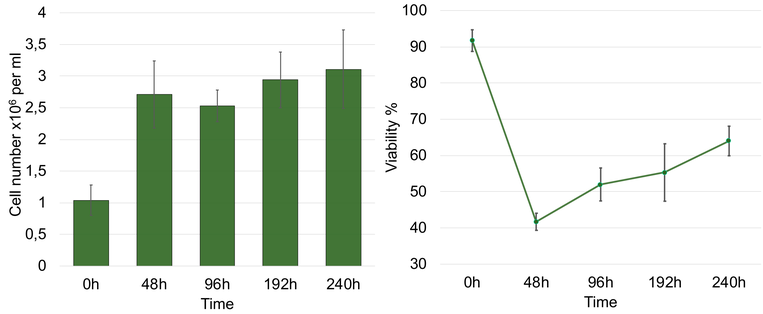
Conclusions

- Cell elongation seen under a microscope could indicate that **cells are interacting with collagen in the gel and acquiring phenotype closer to one seen in living tissues**.
- **Cell number was steadily increasing**, although cell viability within the first 48 hours dropped significantly from 92% to 42%. However, after 96 hours of incubation, it started to increase further and reached 64% after 240 hours. This viability drop can be explained by the stressful encapsulation and temperature changes that cells undergo. Gel dissolving might also be damaging to the cells, so it could also contribute to lower viability values. Other counting and viability assessment methods (such as MTT assay) should be considered for evaluating gel's ability to support cell survival and proliferation.
- The **gels are rigid enough for being moved around and assembled into a more complex structure for irradiation**.
- The **future experiments** include assembling of an elongated gel phantom to study the **relative biological effectiveness of a proton beam along the spread-out Bragg peak**. Another planned study would investigate **the effect of irradiation on a co-culture or mixed culture of normal and cancerous cells** in the same gel.

Results



Pictures taken with inverse light microscope, 40x magnification



Time	Cell number (x10^6) per ml	Viability (%)
0h	1.04 ± 0.24	91.7 ± 2.99
48h	2.71 ± 0.53	41.7 ± 2.36
96h	2.53 ± 0.25	52.0 ± 4.55
192h	2.94 ± 0.44	55.3 ± 7.93
240h	3.11 ± 0.62	64.0 ± 4.08

Cell number and viability of U2OS cells counted at different time points.
Error bars in plots represent standard deviation in absolute values.

Key words

Cell culture; 3D; radiobiology.

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