3DCellMakers: Thermogelling Polymers for 3D Cell Culture

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Introduction

- Goal: To synthesize polymers that provide consistent, reproducible environments for cells to form three-dimensional (3D) multicellular structures.
- 3D Polymer structures are expected to provide representative drug transport and therapeutic characteristics relevant to clinical applications.
- Certain types of inverse thermogelling polymers allow tumor cells as well as non-disease state cells to form 3D spheroid-like structures.
- 3D multicellular structures exhibit characteristic features that are not observed when cells are cultured in 2D. The thermogelling property allows mixing cells with the polymer solution at room temperature before forming a transparent gel at 37 °C.

Methods

- Ethylene oxide sterilized thermogels were dissolved in cell culture medium consisting of DMEM/F12 + GlutaMAX™ basal medium supplemented with 5% (v/v) fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 µg/ml).
- Volume of medium was adjusted for the desired % (w/v) hydrogel, and polymers were dissolved at 2-8 °C for two days.
- Multiple cell lines were used to conduct the experiments.
- In one approach either breast cancer MCF-7 cells co-cultured or not with human fibroblasts CCD-1068SK or human liver carcinoma HEP G2 cells were mixed with cold polymer solution, and the mixtures were transferred into a flat bottom polystyrene multiwall plate (uncoated) and incubated at 37 °C.
- At predetermined time points, pictures of cell cultures were taken. During the culture period, the medium was replaced every 48 to 72 hours.
- In another approach, the polymer solution was pre-warmed in polystyrene multiwall plates and the cell suspension was added on top of the solidified gel. For instance triple negative breast cancer T4-2 cells typically cultured in a serum-free medium with known additives were seeded onto 100 µl of 3DCellMaker gel per well in a 12-well plate and images were taken after two days of culture.

Results

- Three promising polymers for 3D cell culture were identified, and they were named “3DCellMakers®”.
- Poloxamer-hexamethylene diisocyanate poly(ester-urethane) (PEU), poloxamer-methylene diphenyl diisocyanate PEU, and stearate modified methyl cellulose.
- In general, seeding the cells onto the pre-warmed gels allow 3D structures to form quickly (1-4 days), (Fig. 3 & Fig.4)
- Mixing the cells directly with cold polymer solution before heating to gel typically yielded tumors at a later time and of smaller size.
- Tumors ranged in size from 40 µm to 200 µm (Fig. 1,).
- T4-2 cells that are particularly sensitive to their environment for tumor formation also formed 3D structures in less than 48 hours (Fig. 2).
- Co-culture of MCF-7 and CCD-1068SK fibroblasts will require staining to differentiate cell type (Fig. 5)
- AlexaFluor 488 was used to detect E-Cadherin as positive indication of MCF-7 forming tight spheroids (Fig. 6)

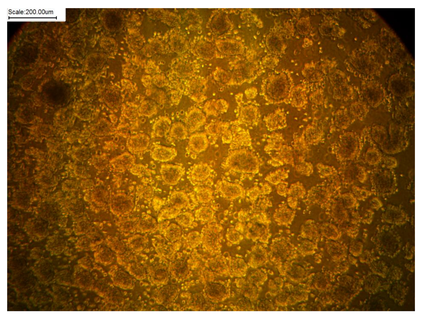

Fig. 1. Vero nodules formed after 4 days of culture.

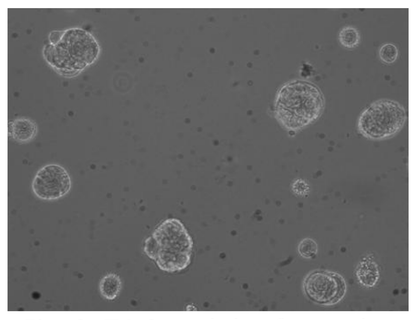

Fig. 2. T4-2 nodules formed after <48 hours of culture.

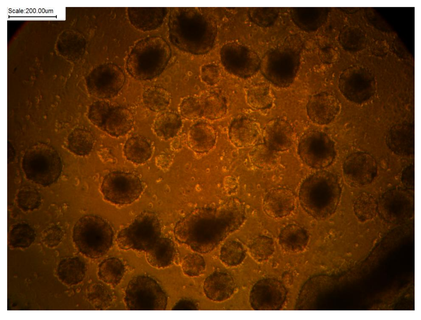

Fig. 3. HEP G2 nodules formed after 6 days culture.

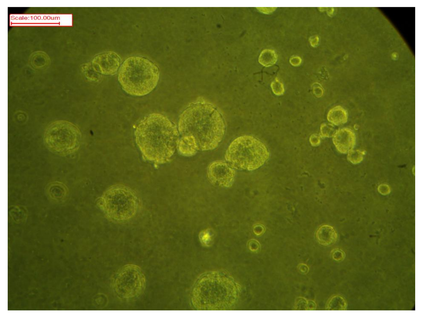

Fig. 4. MCF-7 nodules formed after 2 days of culture.

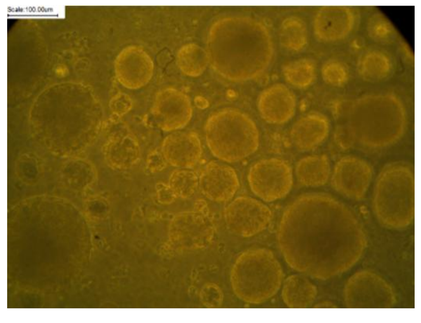

Fig. 5. Co-culture of MCF-7 and CCD-1068SK fibroblast cells.

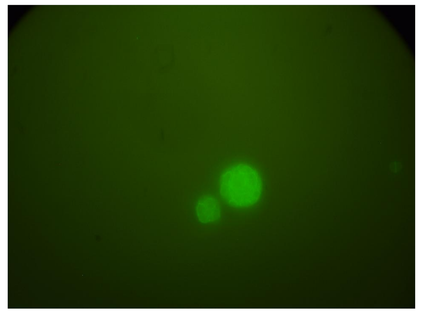

Fig. 6. E-Cadherin/AlexaFluor 488 staining of MCF-7.

Conclusions

- 3DCellMakers have potential to provide an effective, inexpensive, and easy method for generating 3D multicellular structures.
- Thermogelling polymers provide a new avenue of increased productivity in cell biology research for which multicellular 3D structure formation is critical, e.g., studying the efficacy of various drugs and drug delivery systems for treating tumors.
- The ability for cancer cells to form nodules when cocultured with fibroblasts in the polymers provides an interesting avenue to study important aspects of the tumor microenvironment, especially in combination with microfluidic devices or high throughput screening systems.
- Thermogelling polymers allow growth under serum-free conditions of T4-2 invasive cancer cells, which are known to be sensitive to the microenvironmental condition.
- Preliminary results suggest that the 3DCellMaker has potential for broad use.
- Possibility to culture cells with reproducible and known medium conditions will make easier the study of the effect of specific components on tumor growth.

3D Printing Development

- Akina, Inc. is working with Hatch51, A 3D-printer company, on the development of a commercially available printer which can be used for printing 3DCellMaker. Any parties interested in this printing development are encouraged to Contact Hatch51 (http://hatch51.com/)

Acknowledgments

- The patent for 3DCellMakers is Pending.
- This work was partially supported by NCI contract HHSN261201400021C.

Supplemental data

- Available at http://www.3dcellmaker.com/research