COMPARISION OF DIFFERENT EXTRACELLULAR MATRIX IN MCF7 TUMOR SPHEROID FORMATION

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ABSTRACT

Alginate hydrogels have already been extensively used in cell encapsulation, but few investigations have been done to compare cell proliferation and drug response in different extracellular matrices. We previously developed a novel microfluidics approach for tumor spheroid generation and testing: a cell-laden core-shell bead suitable for several days of culture, which contains growing spheroids in varying concentrations of collagen, Matrigel, and alginate within a standardized volume. Here, we compared the growth of MCF7 cells in alginate, collagen-alginate, and Matrigel-collagen-alginate. Alginate gels containing both collagen type I and Matrigel were found to enhance MCF7 proliferation and tumor spheroid uniformity in contrast to alginate alone or collagen-alginate. We used second harmonic generation imaging to visualize the collagen distribution inside the cell-laden beads, and examined dose response in hypoxic conditions.

KEYWORDS: Microfluidics, Flow focusing, Hydrogel scaffold, Tumor spheroid

INTRODUCTION

Extracellular matrix plays an important role in promoting cell proliferation, cell differentiation, and complex cell-matrix and cell-cell interactions. We presented a novel approach for making different matrix embedded 3D beads using microfluidic systems which have shown advantages such as reproducibility, ease of use and uniformity [1]. In this study, we made 3 types of cell-laden core/shell structure beads in which the core composition varied: alginate beads, collagen-alginate beads and Matrigel-collagen-alginate beads. The shell was composed only of alginate in all three bead types. We compared cell morphology, proliferation rate and drug dosage responses between the three types of beads. Within solid tumors, cells can be chronically hypoxic because of limited diffusion of oxygen from blood vessels. Subpopulations of cells within a tumor might become resistant to some drugs because of gene amplification induced by hypoxia [2]. Therefore, we also studied the effect of hypoxia on sensitivity to doxorubicin and tamoxifen in monolayer MCF7 and multicellular MCF7 tumor spheroids.

EXPERIMENTAL

The core/shell structure was formed on the microfluidic chip using multiple inlet flows in a flow focusing geometry as shown in Fig. 1. The first dispersed phase (1) consisted of individual cells dispersed

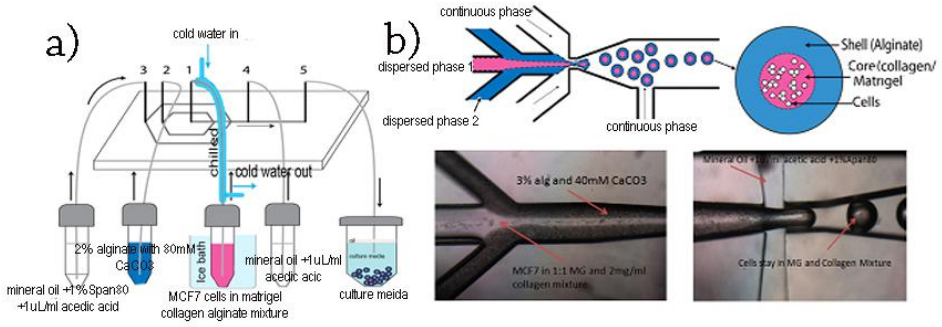

Fig. 1: a) Schematic of microfluidic chip. b) Flow focusing using two dispersed phases and one immiscible continuous phase forms core/shell beads. The cells stay in the bead cores.

in solution of the bead core composition, and was chilled in ice-bath. We used a concentric tube with continuously flowing ice-cold water to chill the collagen/Matrigel/cell suspension to prevent gelation prior to droplet formation on chip. The second dispersed phase (2) consisted of alginate precursor and insoluble calcium carbonate particles. The immiscible continuous phase (3) was mineral oil containing 1 µl/ml acetic acid. Additional acidified mineral oil containing 1 µl/ml acetic acid (4) was added after droplet formation to space the beads to avoid coalescence. The outlet (5) flows to the collection vial, where gel beads were collected. Monolayer MCF7 cells and MCF7 tumor spheroids in beads were seeded into 96 well plates and cultured in air-tight modular hypoxia incubator with a humidified mixture of 5% CO_2 and 3% O_2 as well as a standard normoxic incubator at 21% O_2.

RESULTS AND DISCUSSION

To visualize the collagen fiber distribution in the collagen-Matrigel-alginate beads, second-harmonic generation (SHG) imaging was performed with the Olympus FV1000MPE with a XLPN25XWMP 25x, 1.05 NA water immersion objective lens at an excitation wavelength of 810 nm with a high quality 405/30 emission filter removing any back-propagated signal not originating from the collagen fibers. The observed collagen fibers arranged at the core of the bead (Fig. 2) provide biological scaffolding in which the seeded cells can attach and proliferate into spheroids.

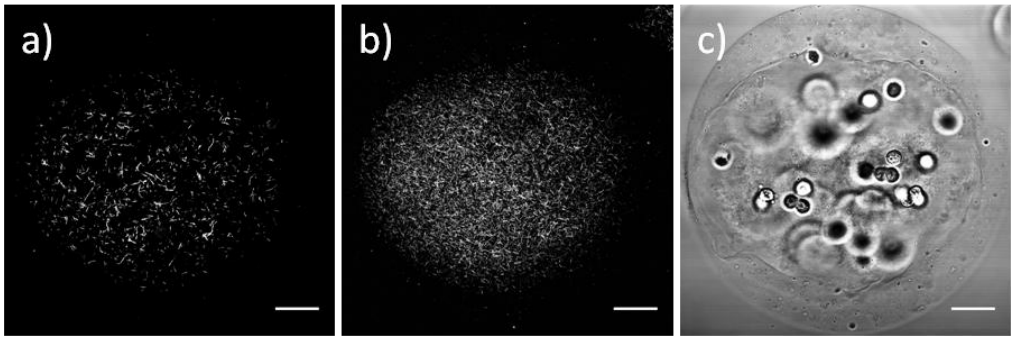

Figure 2: a) Back-propagated SHG image of collagen fibers seeded at the core of a one day old MCF-7 core-shell Matrigel-collagen-alginate bead. The noncentrosymmetric molecular assemblies of collagen fibers allows it to produce bright SHG signals when excited by light in the infrared range. The image was acquired at a depth of 150 µm into the 300 µm thick bead. b) Maximum intensity z-projection of 31 optical sections acquired at 10 µm intervals from the top of the bead to 300 µm into the specimen. c) A differential interference contrast image produced by collecting the forwarded propagated 810 nm excitation light after it passes through the specimen. In this image, the outline of the alginate shell, seeded cells and boundary of the Matrigel-collagen-alginate core can be identified. Each scale bar represents 50 µm.

Fig. 3a shows MCF7 cells 3D morphology in the three bead types at days 1, 4 and 8 after bead generation. The Matrigel-collagen-alginate bead structure can offer much higher control over spheroid uniformity. As shown in Fig. 3 the Matrigel-collagen-alginate MCF7 spheroids exhibited a narrow size distribution with coefficient of variation (C.V.) of only 0.092 compared to alginate beads which is 0.2168. Since cell response to drug treatment may depend on spheroid size, spheroid monodispersity is important in understanding dose dependence. Fig.3b showed that MCF7 cells have a higher proliferation ratio in collagen-alginate mixture and Matrigel–collagen-alginate mixture in contrast to pure alginate. Fig. 4 shows the cytotoxic effects of 24-hour and 72-hour exposure of doxorubicin and tamoxifen to monolayer MCF7 and 3D MCF7 spheroids in hypoxic and normoxic conditions respectively. Collagen-alginate beads showed more drug resistance after hypoxic exposure for 72-hour at high concentration of doxorubicin when compared to normoxic controls. In contrast, tamoxifen did not show different sensitivity in hypoxia or normoxia.

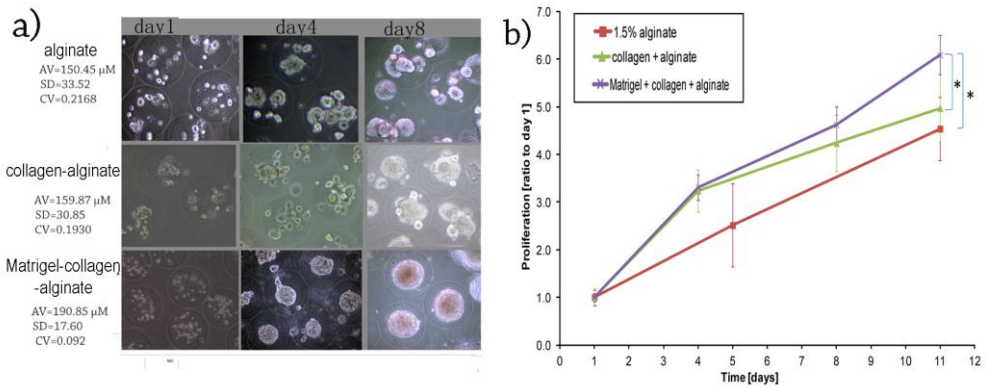

Figure 3: a) 3D morphology of MCF7 cells in the three bead types at days 1, 4 and 8 after bead generation and size distribution of tumor spheroids in each. b): Comparison of MCF7 cell proliferation in alginate beads, collagen-alginate mixture and Matrigel-collagen- alginate beads. Proliferation was measured using a standard MTS assay. ($P < 0.05$)

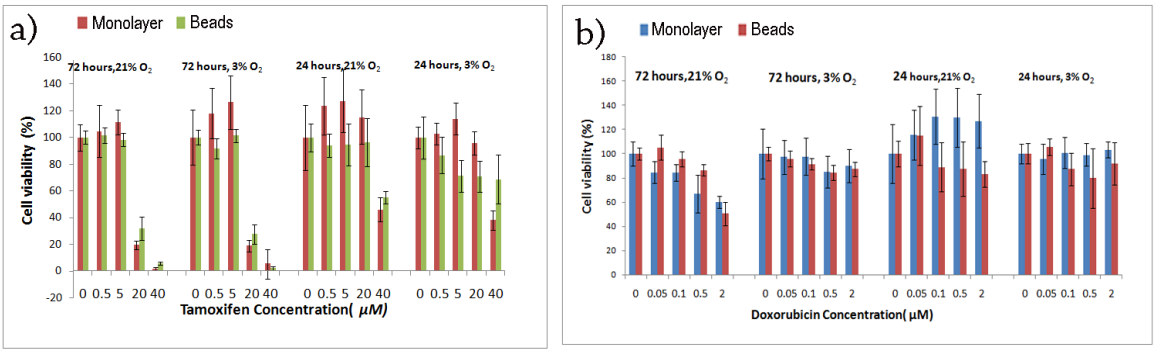

Figure 4: Effects of tamoxifen and doxorubicin on cell viability of monolayer cells and spheroids in Matrigel- collagen- alginate beads in normoxic and hypoxic conditions. The cell viability was verified by MTS assay.

CONCLUSION

The composition of the scaffold affects tumor cell proliferation rate and spheroid uniformity, with the addition of both collagen and Matrigel giving the most uniform sizing. This core/shell bead generation method enables the production of tumor spheroids with a narrow size distribution, providing a more standardized model for future drug screening assays. Future work will investigate the effect of spheroid size on drug dose response in hypoxic and normoxic conditions.

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REFERENCES

[1] L. Yu, C. Bayly and K. C. Cheung. "Matrigel Alginate Core-Shell Beads For Controlled Tumor Spheroid Formation," MicroTAS 2013, 1716, Germany.

[2] C. K. Luk, L. Veinot-Drebot , E. Tjan, "Effect of Transient Hypoxia on Sensitivity to Doxorubicin in Human and Murine Cell Lines," *Journal of the National Cancer Institute*, 82,684(1990)

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