MATRIGEL-AGINATE CORE-SHELL BEADS FOR CONTROLLED TUMOR SPHEROID FORMATION

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ABSTRACT

We present 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 within a standardized volume. The core hydrogel (gelation by temperature control) and shell (gelation by chemical crosslinking) are simultaneously formed through flow focusing. Although spheroid size can influence cell response to drug treatments, current methods generate spheroids with a large size distribution. This novel method enables the production of tumor spheroids with a narrow size distribution, providing a more standardized model for drug screening assays.

KEYWORDS: Microfluidic, Droplet, Hydrogel

INTRODUCTION

Tumor spheroids have become an area of interest for cell-based assay development, due to their superiority in modeling growth and regulatory systems in natural tumors [1]. Microfluidic systems are used to generate three-dimensional hydrogel beads with high throughput and uniformity. Previously, our group employed a microfluidics system to generate and individually monitor regular-sized spheroids encapsulated within hydrogel beads [2]. A shortcoming in this approach has been the tendency of cells to fall or proliferate off the surface of the bead, resulting in rampant 2-D monolayer growth across the culture chamber. Below we describe the fabrication of a collagen and/or Matrigel and alginate core-shell beads, which has been developed to address this issue.

EXPERIMENTAL

FABRICATION OF MICROFLUIDIC CHIP

The microfluidic channels were fabricated using soft lithography with the additional intermediate step of plastic replica molding. High aspect ratio features were first patterned using SU-8 photopolymer on a silicon substrate. These features later defined the microchannels and inlet/outlet reservoirs. The SU-8 and silicon structure served as the mold master, onto which poly-(dimethylsiloxane) (PDMS) was poured. PDMS was cured at 60 °C for 2 h, then the PDMS structure was peeled off the mold master. The droplet formation PDMS structure was then bonded onto a PDMS substrate, and the cell culture chip PDMS structure was bonded to a glass substrate, forming closed channels. Strong bonding was achieved by first briefly treating the PDMS in plasma; the bonded structures were baked in an oven overnight to recover PDMS hydrophobicity. Access to the inlets and outlets were punched through the elastomer and fluidic interconnect was made using syringe needle tips. The schematic illustration of droplet formation chip is shown in Fig. 1.

HYDROGEL BEADS FORMATION

MCF7 cells were maintained in RPMI medium supplemented with 10% fetal bovine serum, and 1% penicillin-streptomycin.

Figure 1: Schematic of microfluidic chip. Inlets to chip (1, 2, 3, 4) are supplied by reservoirs with pressure flow control.

Figure 2: Flow focusing using two dispersed phases and one immiscible continuous phase forms core/shell beads. The cells stay in the Matrigel and collagen mixture in the core of the beads.
The core/shell structure was formed on the microfluidic chip using multiple inlet flows in a flow focusing geometry as shown in Fig. 2. The first dispersed phase (1) consisted of collagen and Matrigel mixed with individual cells dispersed in solution, which was chilled in ice-bath and a concentric tube with continuously flowing ice-cold water was used 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 l/ml acetic acid. Additional, more highly acidified mineral oil containing 2 l/ml acetic acid (4) was added after droplet formation to complete the alginate gelation. The outlet of chip (5) flows to the collection vial, where gel beads were collected. After collection in the outlet vial (5), the gelled droplets were washed in culture media, centrifuged, and placed into standard culture well plates, and the well plates were placed into an incubator (5% CO2, 37 °C). The cells proliferated in the three-dimensional hydrogel environment to form multicellular spheroids. This method produces a core/shell structure in which the shell is composed of alginate, and the core is composed of collagen and/or Matrigel in which cells are embedded.

**EXPERIMENTAL RESULTS**

This new method generates core-shell beads, in which cells were contained in collagen/matrigel mixture and proliferated to form spheroids. Unlike pure alginate core beads, MCF7 cells can break through alginate beads after several days of culture. This reflects the more general challenge presented by alginate bead microculture. High concentrations of alginate are required to keep some cell lines from proliferating outside of the beads. However, high alginate concentrations can also alter the growth behavior of the cell, and high alginate concentration also does not preclude the presence of cells present on the bead surface. Another advantage of collagen/matrigel-alginate core-shell beads is that cell proliferated faster in Matrigel or Collagen than cells in pure alginate and formed around 200 µm compact spheroids in a week which it take much longer for cells to grow the same size of spheroids in alginate. The MCF 7 cells formed 200 µm compact spheroids after 6 days culture (Fig. 3a) in Matrigel collagen mixture beads, but only several small spheroids in alginate after same days culture. This core-shell hydrogel bead structure can offer much higher control over bead/spheroid size, bead/spheroids uniformity. The hydrogel beads exhibited a narrow size distribution with coefficient of variation (C.V.) of only 0.04. Since cell response to drug treatment may depend on spheroid size, spheroid monodispersity is important in understanding dose dependence. Here, the C.V. of the tumor spheroids size is less than 0.09 which is lower than hanging drop or non-adherent substrate methods of generating spheroids (Fig.3b). Fig.3c showed the trapped beads on microfluidic chip. In order to illustrate the cross section and 3D distribution of a tumor spheroid, the spheroids were embedded in resin and sectioned by Leica ultramicrotome at 1 µm interval. The spheroids displayed a solid 3D arrangement of cells using sectioning as shown in Figure 4.

Figure 3: (a) MCF7 cells in alginate or in Collagen/Matrigel (core) Alginate (shell) beads at day 0 and day 6. Cells proliferated quickly and formed spheroids after 6 days culture. (b) Size distribution of beads and tumor spheroids. Bead size and spheroid size were measured after 6 days culture. (c) Cell trapping and culture chip.
After tumor spheroid formation, drug assays of the spheroids were performed immediately by adding different concentration of tamoxifen for 3 days. In this experiment, five concentrations of drugs were treated with tumor spheroids to investigate the significant dose-effect relationship between drug and cell death. These results were also compared with that of the monolayer culture cells. Cell viability was verified by MTT assay. Spheroids of tumor cells have more resistance to tamoxifen than cells grown in monolayer or two-dimensional culture (Fig. 5).

CONCLUSION
This work demonstrates biocompatible core/shell beads which allow spheroids to proliferate and form within the volume of the collagen/ matrigel mixture and prevent the cells to fall outside the beads. In the core/shell method, the core could contain one cell type and the shell could contain a second cell type for three-dimensional co-culture. In future, this method of generating co-cultures as mini tissue constructs also gives advantages in the ability to control size, shape, and uniformity of the constructs compared to conventional methods such as randomly seeding cells into hydrogels in culture wells. These core/shell structures can be generated in an automated fashion at rates of hundreds to thousands per hour, with very high uniformity.

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REFERENCES

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