Abstract

For clinics, and investigation for leukemia, drug sensitivity assay and establishment of cell lines from patients’ samples are important issue. Three-dimensional (3D) cell culture is a promising technique to improve cell survival and function. Here, we developed leukemia culture system and investigated the leukemia cell propagation profiles from patients’ sample with human bone marrow stromal cell using Cell-able system. In addition, we employed insert well system for co-culture with leukemia cells and bone marrow stromal cells, UE6E7T, and 8 patients’ samples in acute leukemia at the diagnosis according the protocol approved by review board and cultured for 56 days. Validation study was also performed using 3 samples. For analyzing the property of the viable cells and the mechanisms of proliferation, we used flow cytometric methods of the viable cells and cytokine array analysis of culture supernatants. The percentage of CD34 positive leukemia cells and the absolute number of leukemia cells was more in separate 3D culture (seeded the bone marrow stromal cells on the microfabricated bottom with culturing leukemia cells in microfabricated insert). Our new culture system was useful for culture with leukemia cells. Especially, easy separation of leukemia cells and stromal cells might make improvement of investigation the properties of leukemia cells.

Background

For clinics, and investigation for leukemia, drug sensitivity assay and establishment of cell lines from patients’ samples are important issue. However, recent gold standard for primary culture system is inoculation to NOD/SCID mice, which is extremely expensive and required large facility. Three-dimensional (3D) sphere”

Methods

Cell-able system consisted of a polystyrene-based plate, which was microfabricated from a block copolymer consisting of a polyethylene glycol (PEG) derivative containing an azide group as a photos cross-linker. Coating the surface of polystyrene with block co-polymer by photolithography inhibits the adhesion of proteins and cells. The non-coated area could accommodate spheroids. In addition, we employed insert well system for co-culture with leukemia cells and bone marrow stromal cells. Stromal cells were cultured on the bottom of the same insert wells or on the bottom of wells in culture plates with or without pattern printing on the bottoms of insert wells and/or wells of culture plates.

Validation study was also performed using 3 samples. For analyzing the property of the viable cells and the mechanisms of proliferation, we used flow cytometric methods of the viable cells and cytokine array analysis of culture supernatants.

Results

1. 3D separate system is least contamination of bone marrow stromal cells.
2. 3D separate system is able to obtain highest percentage of leukemia cells.
3. In terms of Superiority of 3D separate system, G-CSF might be contributed.

Conclusion

Our new culture system, 3D separate system was useful for culture with leukemia cells. Especially, easy separation of leukemia cells and stromal cells might make improvement of investigation the properties of leukemia cells.