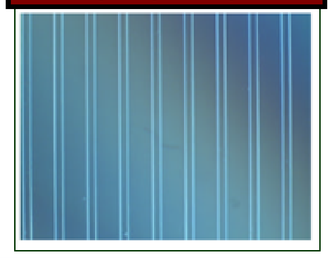
# Abstract

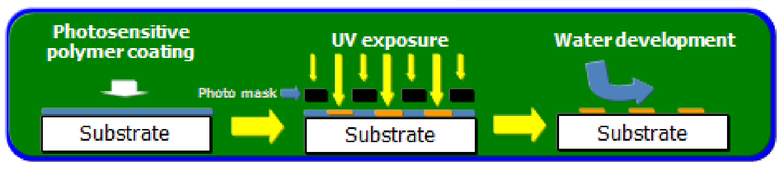
For clinics, and investigation for leukemia, drug sensitivity assay and establishment of cell lines from patients' samples are important issue. Three-dimensional (3D) cell culture is a promising technique to improve cell survival and function. Here, we developed leukemia culture system and investigated 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.

**Photolithographic process to fabricate 3-dimensional cell structure on Cell-able plate**

Chemical surface modification using photosensitive & non cell-attaching polymer.

Example of photolithography (Line pattern)






## Cells

- Leukemia cells
  - All cells were defrosted and used from frozen stocks of bone marrow cells on the initial diagnosis.
  - The protocol approved by review board of Nippon Medical School Chiba Hokusoh Hospital.
- Bone marrow stromal cells
  - Human bone marrow stromal cells, UE6E7T ware obtained from RIKEN.

# Background

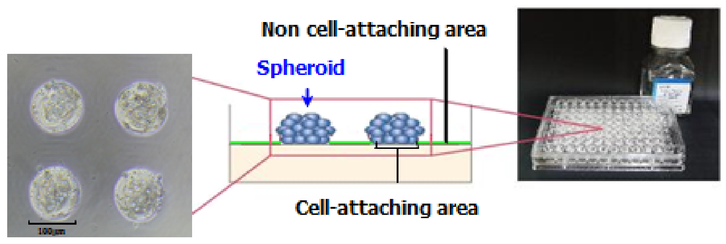
For clinics, and investigation for leukemia, drug sensitivity assay and establishment of cell lines from patients' samples are important issue.

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) spheroid cell culture is a promising technique to improve cell survival and function by preserving cell to-cell interactions. Several groups have reported that 3D spheroid cultures could increase the survival rate of the cells.

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 (TOYO GOSEI Co. Ltd).

**Features of Cell-able**

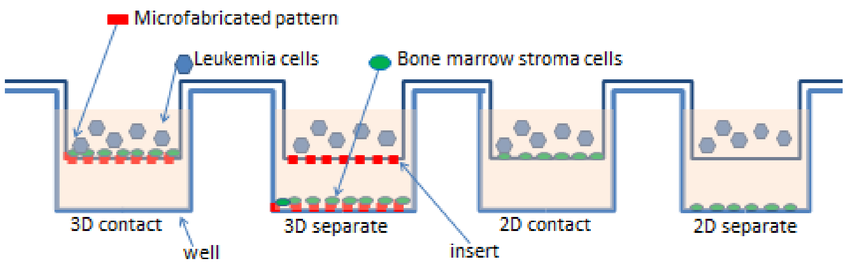



✓Uniform size of spheroids are formed

✓Simple handling for spheroids culture

## Culture pattern

| | Microfabricated pattern | | cells | |
|---|---|---|---|---|
| | on the plate | on the insert | well | insert |
| 3D contact | - | + | - | stroma cells+leukemia cells |
| 3D separate | + | + | stroma cells | leukemia cells |
| 2D contact | - | - | - | stroma cells+leukemia cells |
| 2D separate | - | - | stroma cells | leukemia cells |




# 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 photo 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.

We used human bone marrow stromal cells (UE6E7T), and 8 patients' samples in acute leukemia at the diagnosis according the protocol approved by review board in Nippon Medical School Chiba Hokusoh Hospital 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.

**Transwell system for maintaining long term culture**

Transwell; upper view | Transwell: side view | Transwell: in use

## Culture procedure

- UE6E7T cells (22,000cells/ml)were seeded before starting culture of leukemia cells in POWEREDBY (Glycotechnica, Tokyo, Japan), which was specified to culture of UE6E7T cells.
- After three days culture of UE6E7T cells, culture medium was removed and leukemia cells (2x10(6) cells /ml) were added to culture in RPMI1640 with 10% FBS.
- Culture media were replaced every 2days with 1-2ml.
- Cell count was using Cell Titer-glo (Promega, Tokyo , Japan).

# Results

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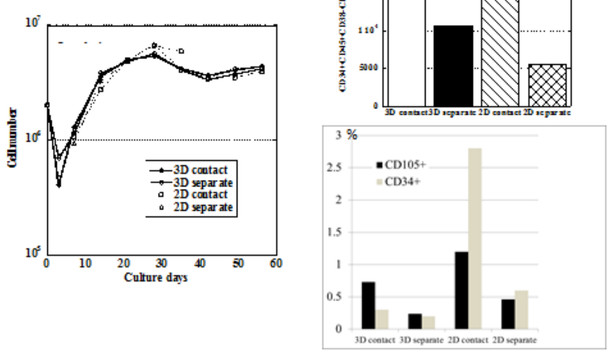





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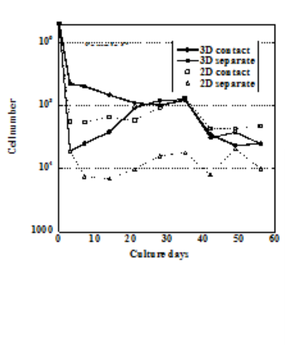


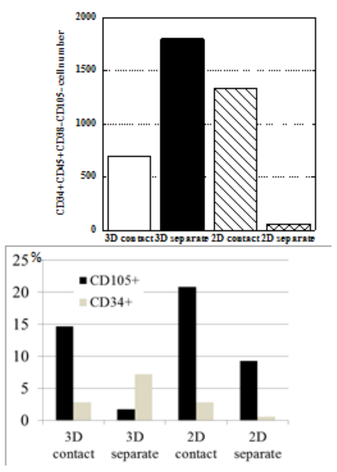


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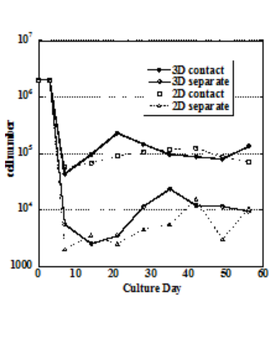


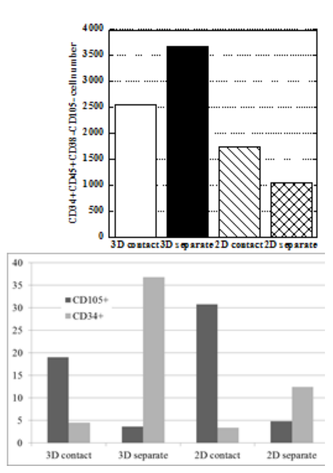


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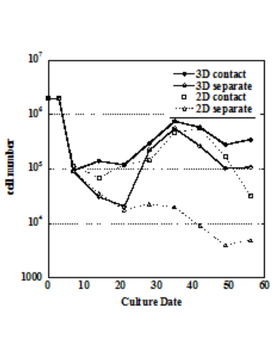


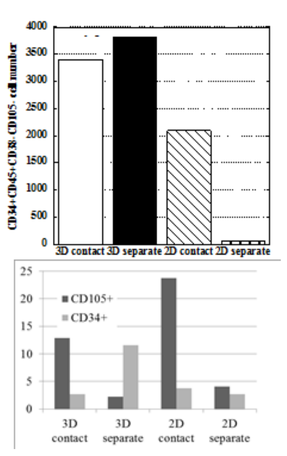


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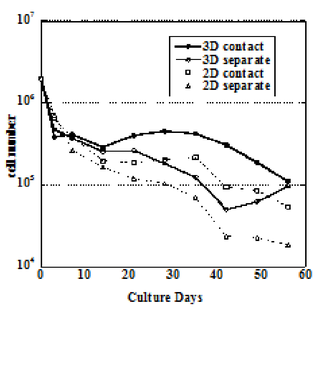


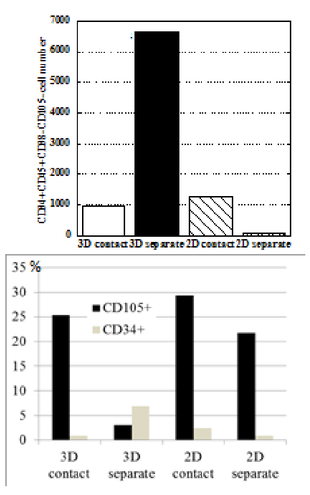


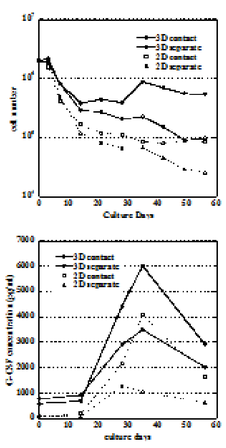


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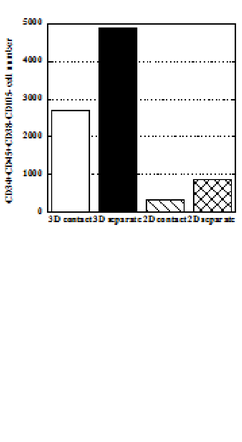


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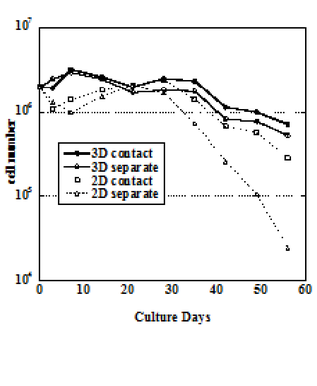
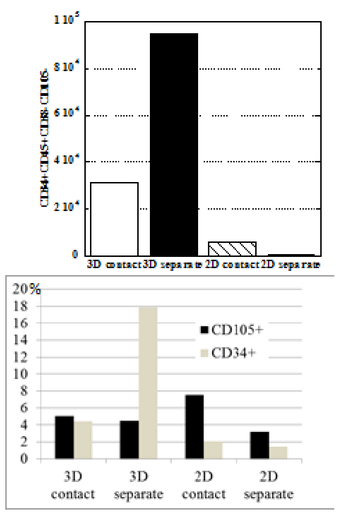
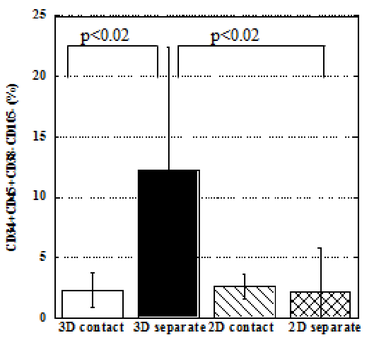
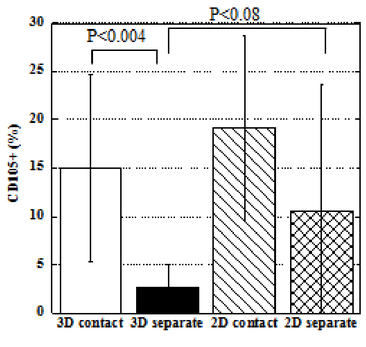



Percentage of CD34+CD45+CD38-CD105-



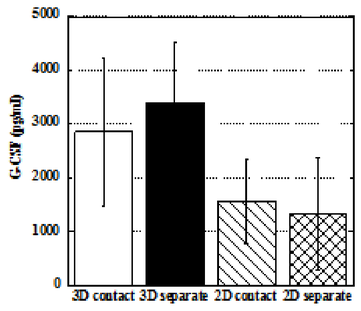

CD105 positive (%)




**Bioplex data from cultured medium**

| | 3D separate | 2D separate | p-value | ratio (3D/2D) |
|---|---|---|---|---|
| IL-1β | 38.1±38.3 | 9.4±2.5 | p=0.323 | 4.08 |
| IL-1ra | 247.8±20.4 | 139.5±7.8 | p=0.005 | 1.78 |
| IL-2 | 3.3±1.0 | 2.5±0.5 | p=0.32 | 1.3 |
| IL-4 | 2.5±0.6 | 2.0±0.1 | p=0.30 | 1.25 |
| IL-5 | 2.9±0.7 | 1.8±0.8 | p=0.27 | 1.57 |
| IL-6 | 7706.0±253.6 | 7436.3±161.9 | p=0.21 | 1.04 |
| IL-7 | 4.8±1.4 | 5.2±1.0 | p=0.70 | 0.92 |
| IL-8 | 13264.1±4574.2 | 6406.6±2759.3 | p=0.105 | 2.07 |
| IL-9 | 6.2±2.4 | 4.3±0.2 | p=0.30 | 1.44 |
| IL-10 | 25.3±4.1 | 24.2±1.3 | p=0.68 | 1.05 |
| IL-12 | 47.5±3.2 | 44.6±3.0 | p=0.31 | 1.07 |
| IL-13 | 2.9±0.3 | 2.5±0.5 | p=0.22 | 1.2 |
| IL-15 | 27.8±1.8 | 28.1±3.3 | p=0.91 | 0.99 |
| IL-17 | 11.5±5.1 | 9.5±1.1 | p=0.56 | 1.21 |
| Eotaxin | 17.2±3.3 | 14.6±0.8 | p=0.30 | 1.18 |
| FGF basic | 155.5±96.7 | 85.2±51.3 | p=0.34 | 1.82 |
| G-CSF | 3224.3±1815.7 | 530.0±257.9 | p=0.01 | 6.08 |
| GM-CSF | 244.0±134.4 | 98.0±14.2 | p=0.20 | 2.49 |
| INF-γ | 79.2±19.5 | 68.1±2.8 | p=0.42 | 1.16 |
| IP-10 | 550.8±500.8 | 687.4±297.6 | p=0.71 | 0.8 |
| MCP-1 | 521.3±27.3 | 588.9±85.0 | p=0.30 | 0.89 |
| MIP-1α | 412.5±636.5 | 33.5±19.9 | p=0.41 | 12.32 |
| PDGF-β | 2.2±0.7 | 1.8±0.1 | p=0.46 | 1.21 |
| MIP-1β | 1052.6±1185.1 | 292.3±388.9 | p=0.33 | 3.6 |
| RANTES | 287.1±313.1 | 996.5±223.0 | p=0.04 | 0.29 |
| TNF-α | 51.0±14.7 | 42.3±2.8 | p=0.41 | 1.21 |
| VEGF | 1911.7±363.7 | 1849.6±294.5 | p=0.83 | 1.03 |

G-CSF data




日本血液学会
COI開示

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■すべての共同発表者を代表し、本発表演題に関連して開示すべきCOI関係にある企業などはありません。

■本研究は日本医科大学千葉北総病院において、IRBの承認を得ている。

Results

1. 3D separate system is least contamination of bone marrow stromal cells.
2. 3D separate system is able to obtain highest percentage of leukemic 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.