

Isolation of Viable Cells in Canine Transmissible Sarcoma (CTS) Using Density Gradient Centrifugation

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ABSTRACT. Density gradient centrifugation using Ficoll-Conray solution was attempted to isolate viable canine transmissible sarcoma (CTS) cells. The viability of isolated tumor cells increased from about 50% to >90%, and the yield of CTS cells was >50% with over 99% purity, in when an isolation solution density of 1.05 was used. — **KEY WORDS:** canine transmissible sarcoma, cell isolation.

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Canine transmissible venereal sarcoma (CTVS) is one of the tumors that can be implanted into dogs. Although many studies have been performed regarding properties of this tumor [1, 3–10, 12–18], some properties of this tumor remain unidentified. Previous studies have used either chemical using trypsin [4] and collagenase [5, 6, 16] or mechanical such as mincing [1, 3, 12, 14, 17, 18] methods for collecting viable tumor cells. Such methods, however, have some disadvantages including lack of cell homogeneity, low cell viability, complex manipulation etc. For further investigation of this tumor, it is necessary to collect more purified and viable tumor cells. Density gradient centrifugation is widely used to isolate lymphocytes from whole blood [2]. We have a experience of CTVS cell isolation using density gradient centrifugation after mincing the tumor [7]. By using this method, it was found to be able to obtain easily viable tumor cells. However, the optimum density of isolation solution and the purity and yield of the tumor cell did not investigated. In this paper, the effects of density of isolation solution on the viability, purity, and yield of the tumor cell were investigated using canine transmissible sarcoma (CTS) derived from CTVS maintained over twenty years by implantation from dog to dog.

Tumor: The 102nd to 105th passages of CTS, that had been maintained by allogenic serial transfer since 1967, were used in this experiment. The CTS originated in a 7-year-old female dog, suffering from canine transmissible venereal sarcoma in Sapporo, Japan [11]. In this study, the CTS at growth and stable stages was used.

Separation Fluid: Ficoll 400 (Pharmacia Fine Chemical, Uppsala, Sweden), a sucrose polymer and, Conray 400 (Daiichi Pharmaceutical Co., Tokyo, Japan), 33.4 % sodium iothalamate in saline, were used as separation media. The separation fluid was prepared by mixing 9.5% w/v Ficoll with 50% v/v Conray in saline to make solutions with densities, 0.01 units apart, ranging from 1.04 to 1.09. The specific gravity of each resulting solution was confirmed using a pycnometer (NRK Co., Ltd., Tokyo, Japan) at room temperature.

CTS Cell Isolation: A crude CTS cell suspension was prepared as previously described [11]. In brief, the tumor

was removed surgically and minced to a brei with scissors. A single cell tumor was obtained by a mechanical crushing technique using a No. 25 stainless steel mesh in a porcelain mortar, and filtered once through a 4-piece gauze. The filtered solution was named a crude CTS cell suspension. Five ml aliquot of a crude CTS cell suspension was placed in a series of 15-ml plastic tubes. The tubes were centrifuged at $200 \times g$ for 10 min at room temperature and then the supernatants were discarded. Five ml aliquots of each Ficoll-Conray density solution were added to individual plastic tubes containing CTS cells and gently mixed. The tubes were centrifuged at $700 \times g$ for 30 min at room temperature. The upper most layer was recovered and transferred to another tube to evaluate CTS cell viability, yield, and purity. Viability of CTS cells was assessed by trypan blue exclusion test. A drop of each the upper most layer was placed on a slide glass for a cytocentrifuge. After air drying, they were fixed in ethanol, stained with Giemsa solution for 20 min at room temperature, and examined morphologically.

Statistical Analysis: Statistical analysis was performed using a Student's *t*-test.

The viability of CTS cell is shown in Fig. 1. Before centrifugation the viabilities at growth and stable stages were $54.9 \pm 10.7\%$ and $43.0 \pm 21.1\%$, respectively. After centrifugation the viability of both stages increased to $90.6 \pm 8.2\%$ and $96.3 \pm 1.8\%$, respectively, when an isolation solution density of 1.05 was used. The higher the density of isolation solution used, the lower the viability of the CTS cells. Cell stage made no significant difference on viability.

The yield of cells is shown in Fig. 2. The CTS cell yield at growth and stable stages was maximum ($56.7 \pm 13.5\%$ and $53.5 \pm 16.4\%$, respectively), when the isolation solution density was 1.06. The yield at a density of 1.04 was significantly lower than that at the density of 1.06, but there was no significant difference between densities of 1.05 and 1.06 as regards cell yield.

The purity of CTS cells was $96.5 \pm 5.0\%$ before centrifugation. That purity increased to >99% after centrifugation regardless of the isolation solution used. Contaminating cells were almost all lymphocytes.

The higher the density of the isolation solution used, the

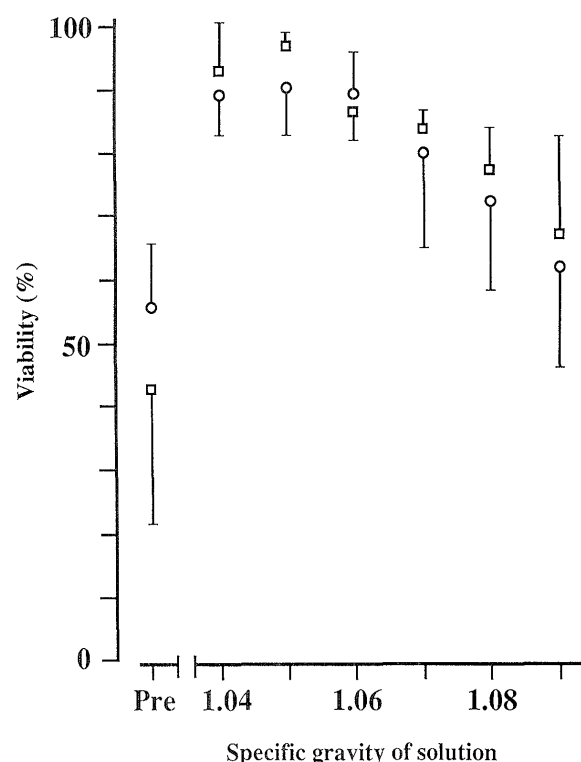


Fig. 1. Correlation between viability of CTS cells and density of separation fluid at growth (○) and stable (□) stages.

more small CTS cells (nuclear diameter from 4 to 5 μ m) isolated regardless of the stages used.

The present study indicates that following mechanical cell preparation an almost pure and highly viable CTS cells can be isolated by density centrifugation using an isolation solution density of 1.05. In general, tumor cells are isolated by either mechanical or chemical methods. In the isolation of CTVS cells, both methods have been used. Although mechanical methods such as mincing [1, 3, 12, 14, 17, 18] are simple to conduct, they result in low cell viability because of mechanical damage. Chemical methods using trypsin [4] and collagenase [5, 6, 16] which result in high cell viability, require complex manipulation, are prone to extraneous cell contamination and are associated with chemical induced surface damage to the tumor cells. Yang and Jones [17] reported that cell contamination was about 20% with the mechanical method. In this study, the purity and viability were >99% and >90%, respectively. These results should facilitate further investigation of the properties and characteristics of CTVS cells.

CTVS cells at each stage have individual characteristics. Yang *et al.* [16] reported that expression of major histocompatibility on the surface of CTVS cells was different between growth and stable stages. Fenton and Yang [6] reported that percentage of positive tumor cells for anti-CTVS antibody was different between growth and stable stages. To investigate property of CTVS cells in detail, therefore, it is necessary to isolate CTVS cells at each stage. In the present result, it was found that cell stage

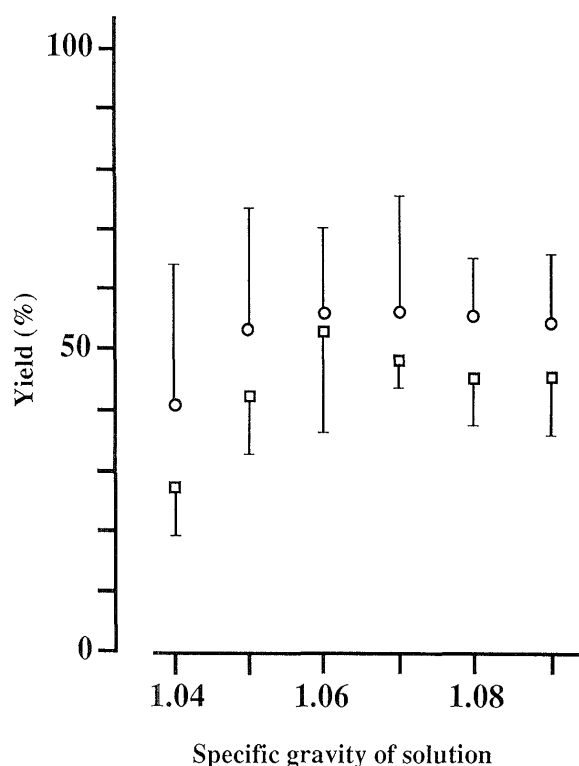


Fig. 2. Correlation between yield of CTS cells and density of separation fluid at growth (○) and stable (□) stages.

made no significant difference on viability, purity, and yield of CTS cells when an isolation solution density of 1.05 was used. This evidence suggests that this isolation method can be used for CTS cells at both growth and stable stages.

Using this optimal condition, isolation of the tumor cell was attempted using 2 cases of naturally occurred CTVS. Those results were consistent with the present one (data not shown).

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