OptiPrep™ Mini-Review MC04

Viable/non-viable cell separation

1. Introduction

Studies of cells from tissues (mammalian and non-mammalian) involve both mechanical and enzymic disaggregation of . for example, liver or spleen or plant leaves, to release single cells. Prior to fractionation of different cell types it is often necessary to remove partially disrupted cells, released organelles and cytoplasm from damaged cells, other often fibrous debris from the tissue and any residual enzymes used in the disaggregation process. It is also good practice to remove non-viable and partially disrupted cells from cell cultures after recovery from cryopreservation.

2. Methodology

2a. Sedimentation mode

In iodixanol solutions, non-viable cells are denser than viable cells and there are two basic strategies for separating out the less dense viable cells, sedimentation or flotation. The often-used Sedimentation Strategy 1, is a simple density barrier (Figure 1). The density of the barrier is chosen to be slightly higher than that of the cells of interest. At the end of the centrifugation the viable cells band and non-viable cells separate acrosss the barrier. The problems with this simple system are: (1) soluble proteins in the sample layer diffuse into the barrier; (2) while most nuclei will probably pellet, most subcellular particles released from disrupted cells will remain in sample layer zone; (3) partially disrupted cells will band across the viable cell layer. In Sedimentation Strategy 2 (Figure 2) a lower density layer (1.05- 1.06 g/ml) is interposed between the resolving layer and the sample. The viable and non-viable cells are again separated across the denser layer but the diffusing soluble proteins will not reach the viable cell layer and there will be less contamination by smaller subcellular particles.

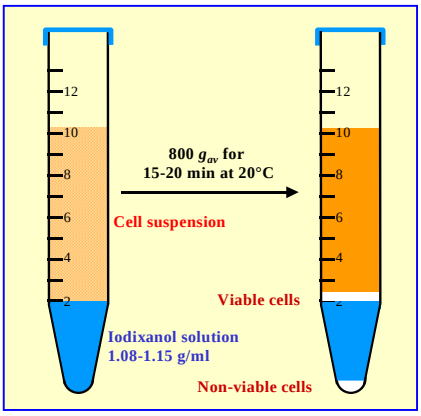

Figure 1: Sedimentation: Strategy 1

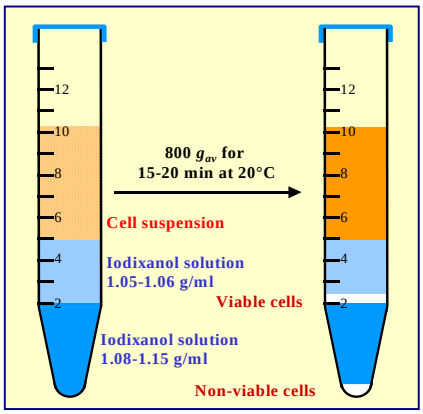

Figure 2: Sedimentation: Strategy 2

In both top-loading strategies all of the particles (cells, non-viable cells, partially broken cells, organelles) will be moving in the same direction and the problems described above will be compounded by the tendency for particles to aggregate at the interfaces. Hence it is usually necessary to wash the recovered viable cells several times to remove contamination by smaller particles and partially-disrupted cells.

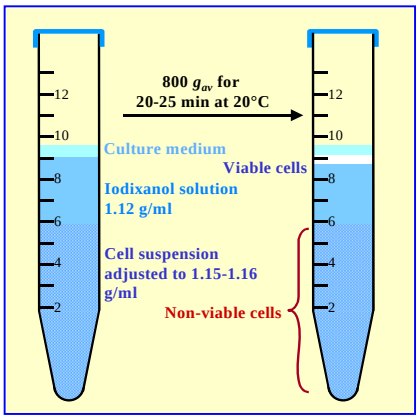

Figure 3: Flotation: Strategy 3

2b. Flotation mode (Strategy 3)

In the flotation mode the sample is adjusted to a density greater than that of the viable cells (Figure 3); this will allow the layering a lower density solution whose density is still greater than that of the viable cells. A small layer of saline is layered on top. During the centrifugation the viable cells float to the top interface; non-viable, partially broken cells and nuclei sediment slowly towards the bottom of the tube. The topmost layer (culture medium or saline) is not critical to the separation but prevents the viable cells from banding at an air/liquid interface. The big advantage of the method is that the viable cells move in the opposite direction to that of the non-viable and partially broken cells and nuclei. Aggregation at interfaces is therefore minimized and there should be layer of clean liquid that is essentially devoid of any contaminants.

2c. Gradient solution preparation

All of the gradient solutions are very easily prepared from OptiPrep™, as sterile 60% (w/v) solution, which makes the preparation of gradient solutions very easy. The methodology using iodixanol gradients for removal of non-viable cells is given in Application Sheet C13. This is accessible from the OptiPrep™ Applications flash-drive or from the following website: www.axis-shield-density-gradient-media.com

Note that although we recommend the OptiPrep™-based flotation strategy (outlined above and described in detail in Application Sheet C13), which should serve as a broadly applicable methodology, there are examples in the literature of the use of technical variations that may have a particular advantage, either for sample handling or for any subsequent technology. For example sedimentation on to a 1.10 g/ml barrier was used for melanoma cells [1,2]; the cell suspension was mixed with an equal volume of 24% (w/v) iodixanol (OptiPrep™ diluted with growth medium) and centrifuged at 800 *g* for 15 min to float viable epithelial cells [3]; HL60 cells were layered on top of three iodixanol solutions of density 1.057, 1.068 and 1.083 g/ml and centrifuged at 450 *g* for 30 min; the viable cells banded at the 1.057/1.068 g/ml interface [4]; a two-step iodixanol gradient (400 *g* for 15 min) was used for progenitor cells [5] and a plasma cell suspension was mixed 3.3:1 with OptiPrep™ (14% w/v iodixanol final concentration) , centrifuged at 1400 *g* for 5 min to float the viable cells [6].

1. Quintana E., Shackleton, M., Sabel, M.S., Fullen, D.R., Johnson, T.M. and Morrison, S.J. (2008) *Efficient tumour formation by single human melanoma cells* Nature **456**, 593-5991
2. Quintana, E., Shackleton, M., Foster, H.R., Fullen, D.R., Sabel, M.S., Johnson, T.M. and Morrison, S.J. (2010) *Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized* Cancer Cell, **18**, 510–523
3. Bruce, A.T., Sangha, N., Richmond, A., Johnson, K., Jones, S., Spencer, T. and Ludlow, J.W. (2010) *Use of iodixanol self-generated density gradients to enrich for viable urothelial cells from non-neurogenic and neurogenic bladder tissue* Tissue Eng., Part C Methods **16**, 33-40
4. Hauert, A.B., Martinelli, S., Marone, C. and Niggli, V. (2002) *Differentiated HL-60 cells are a valid model system for the analysis of human neutrophil migration and chemotaxis* Int. J. Biochem. Cell Biol., **34**, 838-8541282
5. Howell, O.W., Scharfman, H.E., Herzog, H., Sundstrom, L.E., Beck-Sickinger, A and Gray, W.P. (2003) *Neuropeptide Y is neuroproliferative for hippocampal presursor cells* J. Neurochem., **86**, 646-659
6. Chatterjee, M., Stuhmer, T., Herrmann, P., Bommert, K., Dorken, B. and Bargou, R.C. (2004) *Combined disruption of both the MEK/ERK and the IL-6R/STAT3 pathways is required to induce apoptosis of multiple myeloma cells in the presence of bone marrow stromal cells* Blood, **104**, 3712-3721

3. Reference list of papers reporting the use of OptiPrep™

Amphibian cells

Cox, T.C. (1999) *Calcium and ATP regulation of ion transport in larval frog skin* J. Comp. Physiol. B **169**, 344-350

Bacteria

Dehusa, O., Pfitzenmaier, M., Stuebs, G., Fischer, N., Schwaeble, W., Morath, S., Hartung, T., Geyer, A. and Hermann, C. (2011) *Growth temperature-dependent expression of structural variants of Listeria monocytogenes lipoteichoic acid* Immunobiology, **216**, 24–31

Bronchoalveolar lavage cells

Kotzin, J.J., Spencer, S.P., McCright, S.J., Uthaya Kumar, D.B., Collet, M.A., Mowel, W.K., Elliott, E.N., Uyar, A. et al (2016) *The long non-coding RNA Morrbid regulates Bim and short-lived myeloid cell lifespan* Nature, **537**, 239-243

Breast tumour cells

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Colonic macrophages
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Steinbrunn, T., Chatterjee, M., Bargou, R.C. and Stühmer, T. (2014) *Efficient transient transfection of human multiple myeloma cclls by electroporation – an appraisal* PLoS One, **9**: e97443

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Hepatocytes (see also "Human foetal liver cells")
Schmelzer, E., Wauther, E. and Reid, L.M. (2006) *The phenotypes of pluripotent human hepatic progenitors* Stem Cell.,**24**, 1852-1858
Sicklick, J.K., Li, Y-X., Melhem, A., Schmelzer, E., Zdanowicz, M., Huang, J., Caballero, M., Fair, J.H., Ludlow, J.W., McLelland, R.E., Reid, L.M. and Diehl, A.M. (2006) *Hedgehog signaling maintains resident hepatic progenitors throughout life* Am. J. Physiol. Gastrointest. Liver Physiol., **290**, G859-G870

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Våtsveen, T,K., Børset, M., Dikic, A., Tian, E., Micci, F., Lid, A.H.B., Meza-Zepeda, L.A., Coward, E. et al (2016) *VOLIN and KJON-Two novel hyperdiploid myeloma cell lines* Genes Chromosomes Cancer, **55**, 890–901

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Liu, S.J. (2013) *Characterization of functional capacity of adult ventricular myocytes in long-term culture* Int. J. Cardiol., **168**, 1923–1936

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