Axis-Shield Mini-Review MC06

Dendritic cells from blood and tissues

This Mini-Review primarily provides a complete bibliography of publications that report the use of OptiPrep™ for the purification of dendritic cells (DC) from blood and tissues (Section 2). It also provides a brief review of the methodological options for the gradient separations (Section 1). All Application Sheets can be accessed on the Axis-Shield Applications flash-drive or via the Axis-Shield website www.axis-shield-density-gradient-media.com. Click on "Methodology" and then "Mammalian and non-mammalian cells" and follow the links from the Index.

1. Methodological review

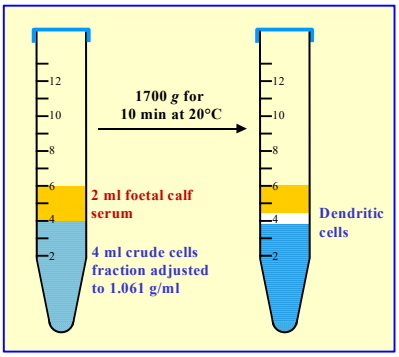
1a. Sedimentation on to a barrier

Prior to the introduction of Nycodenz®, gradients of either albumin or metrizamide were commonly used in DC purification. These media however tended to cause functional alteration of the cells [1]. Because cells are more tolerant of Nycodenz®, after 1994 this iodinated density gradient medium rapidly became established as the medium of choice for DC purification from peripheral blood and from lymphoid tissues. Density barriers of ρ = 1.076 g/ml to 1.088 g/ml [2-7], the majority being approx 1.077 g/ml, have been used in the "traditional" format in which the crude cell fraction is layered on top. DCs band at the interface and further enrichment is often carried out by negative selection with antibody-bound beads. McLellan et al [8,9] introduced a lower density of 1.068 g/ml for DC isolation from human peripheral blood mononuclear cells, depleted in T-lymphocytes by rosetting with neuraminidase-treated sheep erythrocytes. Use of this medium has also been extended to tissues [10,11]. McLellan et al [8] noted that the 1.068 g/ml barrier was also effective for removing denser T-lymphocytes. This strategy of layering the cell suspension on top of a barrier has been extended to the use of OptiPrep™ for isolation of DCs from various tissues (12-17). The barrier sedimentation method is described in the Axis-Shield Application Sheet C47.

1b. Flotation methods

Generally speaking isolation of the least dense particle from a mixture of predominantly denser particles (and this is essentially what is happening in the isolation of DCs), is better accomplished by flotation rather than sedimentation. If the cells are suspended in a solution whose density is slightly higher than DCs, the latter will float to the top while the denser cells will either remain suspended in, or sediment slowly to the bottom, of the suspending medium. An important facet of this strategy is that avoids the rapid accumulation of cells, and consequent aggregation, that occurs during sedimentation on to a density barrier. This strategy was first introduced by Shortman's group in 1996 [18-21], in which the cells from spleen, thymus, lymph nodes etc were suspended in a solution of Nycodenz® (ρ = 1.077 g/ml). This approach was adapted by Anjuere et al [22] to OptiPrep™ in 1999 and there has been tendency towards the use of lower densities for the suspending solution (e.g. 1.061 g/ml) to improve the purity of the DC harvest. This method (see Fig. 1) is described in the Axis-Shield Application Sheet C21.

A modification to this flotation format is to suspend the cells in a medium of higher density, which is placed below the separating solution of ρ = 1.065 g/ml [23]. This is a commonly used format for the selective isolation of a low density cell type; its advantage is that the band of cells is divorced from the crude fraction layer, which is likely to contain residual tissue dispersing enzymes and components from partially disrupted cells. This method (see Fig. 2) is described in the Axis-Shield Application Sheet C20.



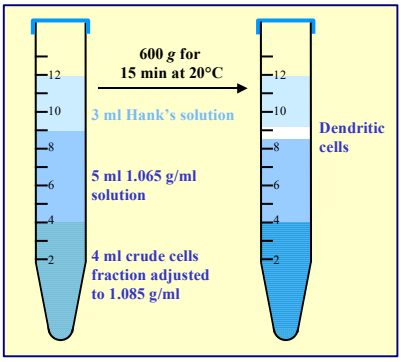


Fig. 1 (top) C21 method
Fig. 2 (bottom) C20 method
See text for details

1c. References (to Section 1)

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2. OptiPrep™ bibliography

References are divided into sections (**Sections 2.1-2.20) a**ccording to **tissue source** and, where the number of references demands, sorted further into **research topic** sub-sections. Within each section references are listed alphabetically according to first author. This bibliography was last updated in September 2017.

2.1 Blood

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2.2.5 Lipopolysaccharide stimulation

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