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Percoll Gradient Optimization for Blood CD133+ Stem Cell Recovery

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Abstract

Circulating CD133+ stem cells from the peripheral blood have been shown to be able to differentiate into numerous cell lineages. However, adults have only a small number of these circulating stem cells. The aim of the present study was to assess a new isolation and enrichment technique for CD133+ stem cells from peripheral blood with the use of Percoll density gradients. Our results demonstrated the presence of two large mononuclear bands when whole blood was centrifuged with 48% and 50% Percoll concentrations. Flow cytometric analysis (FACS) revealed a major CD133+ enrichment at the 48% Percoll concentration in one of the two bands. Further culture of these cells resulted in the formation of multiple colony-forming units. Our results suggest an advantage from using a simple Percoll gradient for successful CD133+ cell recovery, which could aid in differentiation and transplantation protocols.

Keywords

Stem Cells Recovery, CD133+, Percoll, Density Gradients

1. Introduction

There are some multipotent adult stem cells in the bone marrow. These cells have the capacity to differentiate into numerous cell lineages, such as cartilage, bone, fat, muscle, neuron and tendon [1]-[6]. This fact makes them attractive candidates for cell and gene therapy.

It has been shown that stem cells are also resident in other tissues, including blood [7], which raise the possibility of having an accessible and less traumatic source. In addition, peripheral blood stem cells are known to be capable of regenerating damaged or diseased tissues [8] [9].

Stem cells express CD133 antigen [10] [11]. CD133, therefore, is an antigen that could serve as an alternative

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to CD34 for the selection and expansion of stem cells for transplantation and differentiation into several cell lineages. The CD133+ progenitors have been shown to be capable to form colony-forming units (CFUs) *in vitro*, and transplantation into NOD/SCID mice resulted in a high engraftment rate with conservation of its multilineage capacity [12]-[14]. CD133+ progenitors cells have also been used for autologous transplantation [15] [16] and demonstrate its capability to differentiate into motor preneurons *in vitro* [17].

Percoll has been widely used to selectively separate cells by their density characteristics. It has also been shown to be efficient in separating mononuclear cells from erythrocytes out of whole blood samples [18], as well as from bone marrow [19] and umbilical cord blood [20]. Additionally, it has been used to isolate many cell organelles [21].

The aim of this study was to optimize the use of Percoll gradients for the isolation of CD133+ stem cells from peripheral blood samples from adult.

2. Material and Methods

2.1. Blood Samples

Fifteen-milliliter samples of peripheral blood were obtained by venipuncture from healthy human adult volunteers. Heparin was used as an anticoagulant. The blood was diluted 1:2 with phosphate buffer saline (PBS) and 2 mL was carefully added to a 1 mL Percoll gradient.

2.2. Percoll Gradients

The cells were fractionated on a discontinuous density gradient prepared with the following Percoll (Research Organics, Cleveland, OH, USA) concentrations: 70%, 65%, 50%, 48%, 46% and 44% in PBS. After centrifugation (450 × *g* for 30 min) the resulting bands were collected and washed twice with PBS. A Lycoprep sample was used as a control.

2.3. Density Determination

Density gradient determination was assessed using a 9.901 cm^3 pycnometer (Brand, Germany) calibrated at 17˚C. Determinations were taken at 18˚C. All determination was performed by triplicate.

2.4. Flow Cytometric Analysis

The total blood cell contents of the different bands recovered were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD34 (HPCA-2-FITC; Becton Dickinson, New Jersey, USA) and phycoerythrin (PE)-conjugated anti-AC133 (Miltenyi Biotec Bergisch Gladbach, Germany) for 60 min on ice in the dark. After incubation, the cells were washed once in PBS. Cell sorting was performed on a FACS Vantage (Becton Dickinson) equipped with an argon laser tuned to 488 nm. Determinations were performed by triplicate.

2.5. Cell Culture

Cells from the recovered bands were incubated in separate Petri dishes with DMEM-F12 media supplemented with 10% fetal bovine sera (FBS) at 37˚C in 5% CO_2 in air. The cultures were followed with an inverted microscope.

3. Results

Density Determination

All densities analyzed formed a mononuclear band with good resolution. **Table 1** shows the density determination performed on the different Percoll concentrations used, as well the band number formed.

Densities of 48% and 50% gave the best resolution, in that a wide band (b) formed between the erythrocytes (a) and a second thin band (c) below the serum fraction (d) (**Figure 1**).

Data from FACS showed a high number of $CD133^+$ as well $CD34^+$ cells in band (b). At 48% Percoll, corresponding to 1.06112 (**Figure 2**).

FACS analyses demonstrated a higher number of CD133+ cells compared with the Lycoprep as control and

Table 1. Density from different Percoll concentration and number of bands recovery.

Percoll Concentration	Density	Bands Recovery
46%	1.0571	1
48%	1.06112	2
50%	1.06294	2
60%	1.075411	
70%	1.0889	1
Lycoprep	1.077	1

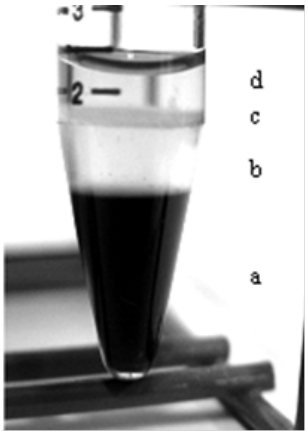

Figure 1. Photography of 48% Percoll concentration bands. (a) erythrocyte pellet; (b) wide mononuclear band; (c) thin mononu-clear band; (d) sera band.

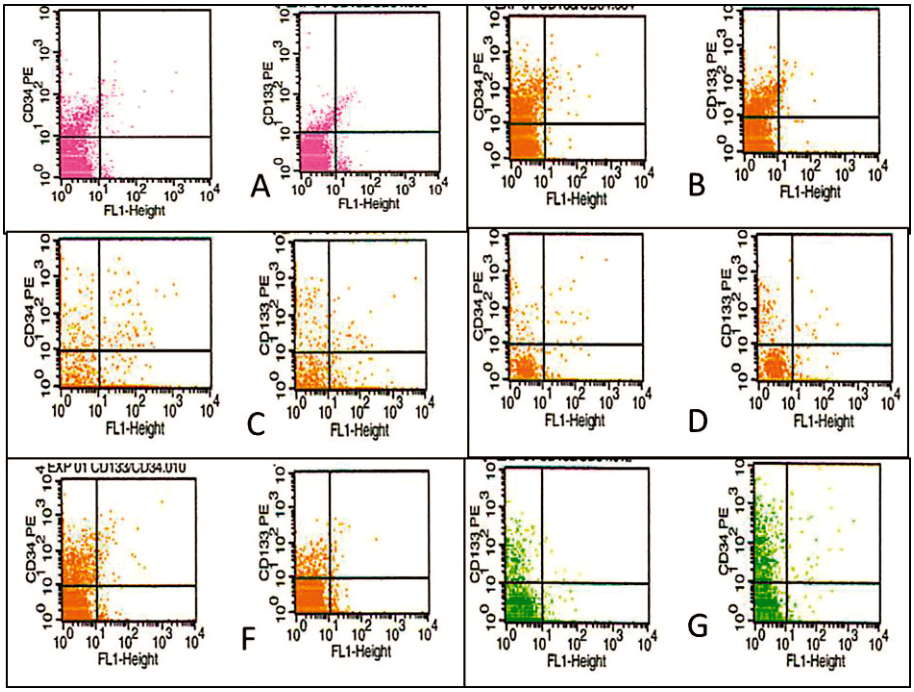

Figure 2. FACS of CD34+ and CD133+ cells analisys on total blood and different concentration of Percoll density. (A) total blood; (B) Lycoprep; (C) 46%; (D) 48% Percoll; (E) 50 %; (F) 60% Percoll.

with the other Percoll concentration. The percentages of CD34+ and CD133+ cells recovered are shown in **Table 2**.

All cells isolated with the use of the 48% Percoll concentration produced viable cultures (**Figure 3**) with multiple CFU.

4. Discussion

Percoll at 70% concentration is prevalent in stem cell separation protocols [22]. This concentration corresponds to a 1.0889 density, which is higher than that reported for mononuclear cells. However, the density of l.075 obtained from a 60% Percoll concentration, is similar to that obtained from commercial preparations. This concentration gave a good resolution for CD34+ cells, but not for CD133+ cells [23]. The median percentage of CD133+ recovery with this approach is 0.75% (range 0.39% - 2.03%) [12]. Our results show that a 48% Percoll concentration gave 12.62% recovery of CD133+ cells. These data represent an important difference for protocols aimed at transplantation and/or differentiation. It has been shown that CD133+ cells are the choice in many instances, because of their lower conversion to malignancy [15]. Furthermore, CD133+ cells have been shown to present a higher plasticity for differentiation into endothelial cells [23] and neurons [15] [24].

5. Conclusion

The use of 1.061 densities (48% Percoll) for gradient separation of CD133+ cells from peripheral blood, gives better yields than the use of 1.077 densities, commonly used for gradient isolation of stem cells, in particular those which carry the Antigen CD133+.

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Table 2. Percentage recovery of CD34+ and CD133+ cells from different band b Percoll concentration.

Percoll Concentration	%CD34+	%CD133+
Total blond	7.05	2.68
Lycoprep	12.34	7.70
Percoll 46%	12.87	11.27
Percoll 48%	11.53	12.62
Percoll 50%	6.84	5.35
Percoll 60%	15.58	4.03

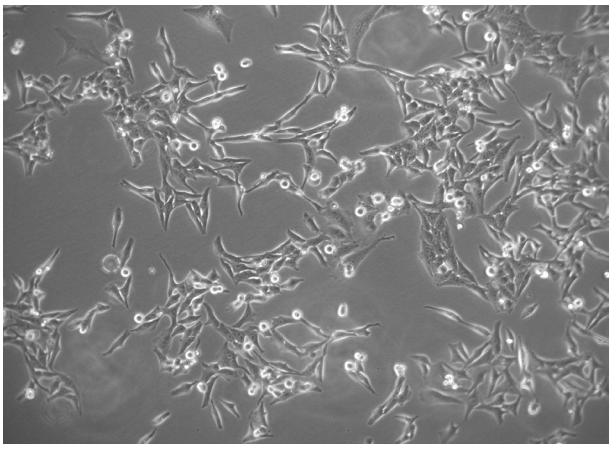

Figure 3. Microphotography of cells incubated with DMEM-F12 medium after 20 days recovery from 48% Percoll concentration. Multiple rounded mitotic cells are observed.

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