Simplified Neutrophil Isolation Protocol

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Abstract

Background: Neutrophils are the most abundant leukocytes in humans and among the first cells to arrive on the site of inflammatory immune response. Due to their key role in inflammation, neutrophil functions such as locomotion, cytokine production, phagocytosis, respiratory burst and release of extracellular traps are extensively studied.

Objective: Develop a simple, fast and cheap method to isolate neutrophils from human blood.

Methods: A blood sample of 15 ml was obtained from healthy subjects. Subsequently, neutrophils were obtained using the modified density gradient separation method. Finally cell viability and morphology was analysed.

Results: An average of 1.5 × 10⁷ cells/ml was obtained with a > 98% neutrophil purity and a viability greater than 95%.

Conclusions: Our protocol demonstrates that neutrophils can be isolated without investing a lot of money per procedure, which means this protocol will simplify the struggle of scientists to isolate neutrophils in terms of steps and expensive material.

Keywords
Neutrophil, Protocol, Gelatin

Introduction

Neutrophils are the most abundant leukocytes in humans and among the first cells to arrive on the site of inflammatory immune response. Due to their key role in inflammation, neutrophil functions such as locomotion, cytokine production, phagocytosis, respiratory burst and release of extracellular traps are extensively studied. In the present study, we present a simplified neutrophil isolation protocol which has excellent reproducibility and low cost. Taken together, our findings point to the fact that this protocol can be used in any lab around the world, but is mainly focused in labs with low income that wish to isolate neutrophils for their studies.

Methods

Study population

Healthy subjects (HS) were recruited at the Family Medicine Department of the University Hospital “Dr. José E. González”. All procedures were reviewed and approved by the Research and Ethics Committee of our institution with the number IN18-0005. All participants provided a written informed consent.

Neutrophil isolation protocol

1. Extract 15 ml of peripheral venous blood using tubes with EDTA.
2. Place 7.5 ml of sedimentation fluid (3% Gelatin [Difco Gelatin 214340], 0.9% NaCl and 0.1% Dextrose at the bottom of a 50 ml conical tube. (1:2 ratio).
3. Place the 15 ml of venous blood over the sedimentation fluid and mix by inversion 3 times.
4. The tubes will we kept at room temperature for 15 min to allow the red blood cells to sediment.
of 1.5 × 10^7 cells/ml was obtained with a > 98% neutrophil purity and a viability greater than 95% (n = 15) (Figure 2 and Figure 3).

**Discussion**

**Isolation of neutrophils**

Different methodologies for the isolation of neutrophils were tested [1-6], but the best method we found to isolate neutrophils was the one described by García-García, et al. [7].

The density gradient separation method reported by García-García, et al. provided the best method to...
In terms of cell viability and number of neutrophils obtained per procedure, Dextran and Gelatin obtain similar results, although gelatin takes only 15 min to sediment red blood cells while dextran takes 30-60 min, which makes our protocol suitable for labs with low income that wish to isolate neutrophils for their studies.

**Conflicts of Interest**

We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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Gelatin has a molecular weight of 100,000 to 120,000, this macromolecule helps aggregate red blood cells and thus is able to sediment them very fast. Red blood cell aggregation represents a balance of forces at the cell surfaces, and aggregation results when the macromolecular bridging force exceeds the electrostatic repulsive force and the mechanical shearing force. The larger a macromolecule is, the more effective it is at inducing red blood cell aggregation because of a weaker electrical repulsion due to the longer intercellular distance, and a stronger bridging force due to the larger adsorption area on the cell surface [8-10].

![Figure 3: Neutrophils stained with methylene blue 0.1%](image-url)