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Isolation and Characterization of Small Pluripotent Stem Cells (SPSCs) from Human Peripheral Blood: A Novel Cold-Enrichment Protocol

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Abstract

Introduction:
Small Pluripotent Stem Cells (SPSCs) represent a novel population of blood-derived stem cells exhibiting key pluripotency markers (OCT4, NANOG, SOX2, SSEA-4), measuring <5 μm in size. Isolated through a non-manipulated, cold-enrichment protocol from peripheral blood, SPSCs demonstrate nuclear integrity, high viability, and potential for both local and systemic therapeutic delivery. Unlike larger stem cells such as mesenchymal stem cells (MSCs), which are often sequestered in pulmonary capillaries, the small size of SPSCs may allow for wider biodistribution. Their autologous origin and compliance with FDA and EMA minimal manipulation guidelines make them an ideal candidate for future regenerative applications. Methods: Peripheral blood samples (10 ml) were collected from healthy adult volunteers n=233 total, (200 for baseline, 23 for enrichment, 10 for staining) to be centrifuged according to the SBSC protocol published by Filidou et al. (2023). Cell concentration and viability were measured before and after cold storage using the LunaStem™ automated cell counter. Immunocytochemistry was performed using the Invitrogen™ Pluripotent Stem Cell Immunocytochemistry Kit (OCT4, SSEA-4, NANOG, SOX2). Images were captured using a Magus Lum D400 fluorescence microscope with MAGUS CLM30 digital camera.

Results:
LunaStem™ analysis showed that the total nucleated cell concentration increased from ~20 million/ml to ~40 million/ml after 4.5h in cold storage, most prominently in the 1–3 μm size range. In multiple independent experiments for enrichment (n=23), a consistent ~2–3-fold increase in viable small cells (~1 μm) was observed. Pluripotent markers from separate blood samples (n=10) were shown and presented using immunofluorescence microscopy.

Conclusion:
This study introduces a novel, minimally invasive method for isolating and enriching Small Pluripotent Stem Cells (SPSCs) from human peripheral blood. We observed a statistically significant 2-fold increase (p=0.0001) of pluripotent cells with yields ranging from 200–500 million SPSCs from 60 ml whole blood.

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