

Expansion and Characterization of Cord Blood-Derived CD34+ Cells on NANEX™ 3-D Nanofiber Scaffold

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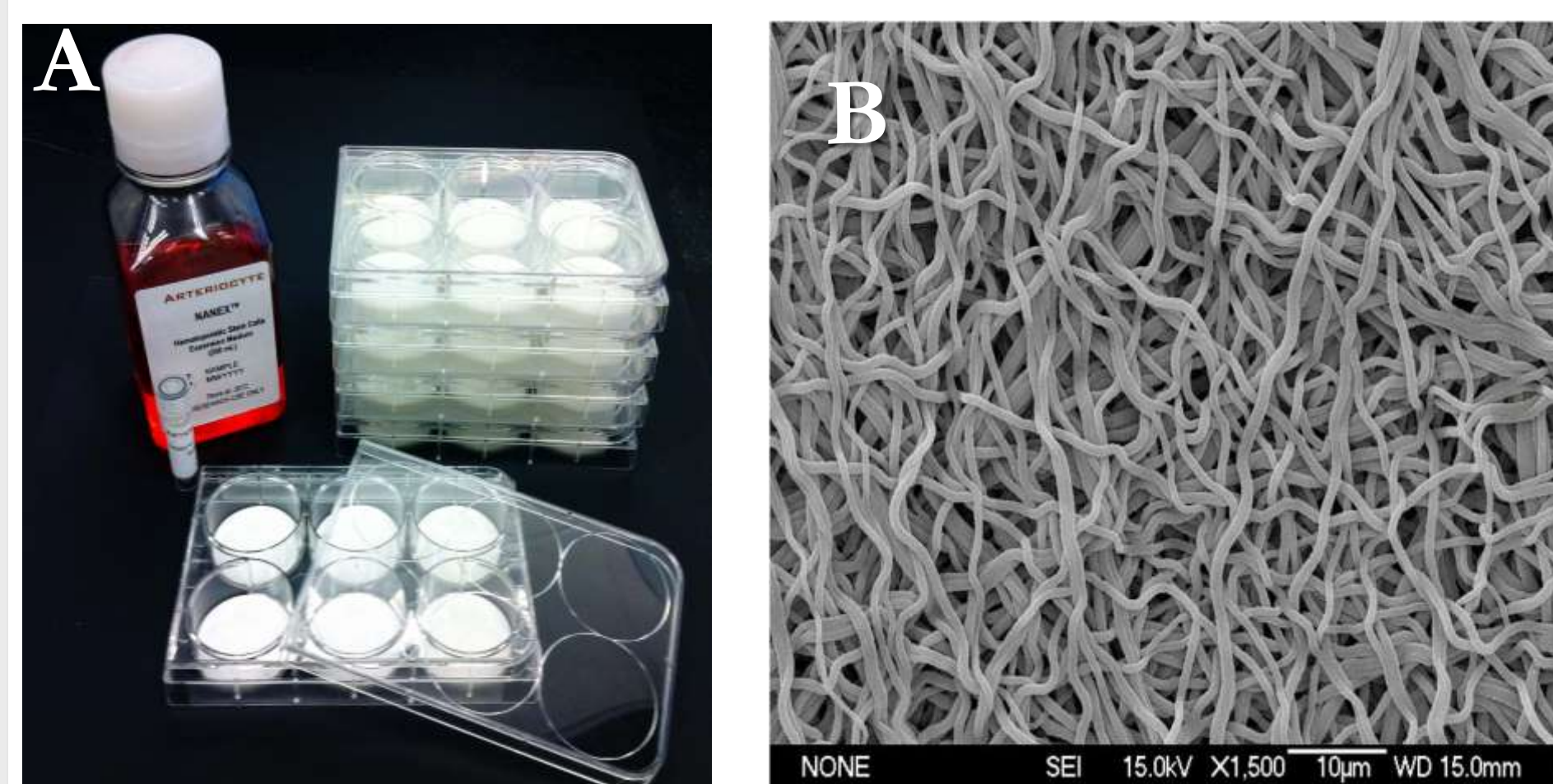
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INTRODUCTION

In recent years, umbilical cord blood (UCB) has become recognized as an important source of hematopoietic stem cells (HSCs). However, the absolute number of HSCs is significantly lower in UCB than it is in bone marrow or mobilized peripheral blood, which has limited the broader use of cord blood in numerous applications. Arteriocyte Inc. has recently developed a 3-D nanofiber cell culture substrate (NANEX™) designed to provide topographical and substrate-immobilized biochemical cues that act in synergy with media additives to enhance hematopoietic stem and progenitor cell (HSC/HPC) proliferation. Our data indicates that NANEX™ technology provides a robust *ex vivo* expansion of UCB-derived HSCs/HPCs and offers great promise for increasing the use of cord blood as an HSC source.

BACKGROUND

The NANEX™ Stem Cell Expansion System, which consists of NANEX™-coated culture dishes (6-well or 24-well), serum-free medium, and an optimized cocktail of growth factors, is designed for the efficient *ex vivo* expansion of HSCs/HPCs from bone marrow, peripheral blood, and umbilical cord blood. The NANEX™ cell culture substrate is completely synthetic, and is produced by a two step process consisting of (1) electrospinning of nanoscale polymeric fibers followed by (2) surface treatment of the fibers to enhance cell adhesion. When used in combination with Arteriocyte's HSC medium and growth factor cocktail, NANEX™ can facilitate the proliferation of HSCs/HPCs with significantly reduced stem cell phenotype loss.



(A) NANEX™ Stem Cell Expansion System, (B) Scanning electron micrograph of NANEX™ nanofiber substrate (1500X magnification).

HYPOTHESIS

In the natural bone marrow microenvironment, HSCs maintain close contact with a complex network of stromal cells and extracellular matrix, likely indicating that cell-cell and cell-matrix interactions play an important role in maintaining their stem cell phenotype. Since the NANEX™ substrate partially mimics this stem cell niche (by way of its 3-D topography and substrate-immobilized biochemical cues), it can facilitate the proliferation of HSCs/HPCs to a greater extent than traditional flat substrates.

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METHODS

CD34 Isolation from Umbilical Cord Blood and Ex Vivo Culture:

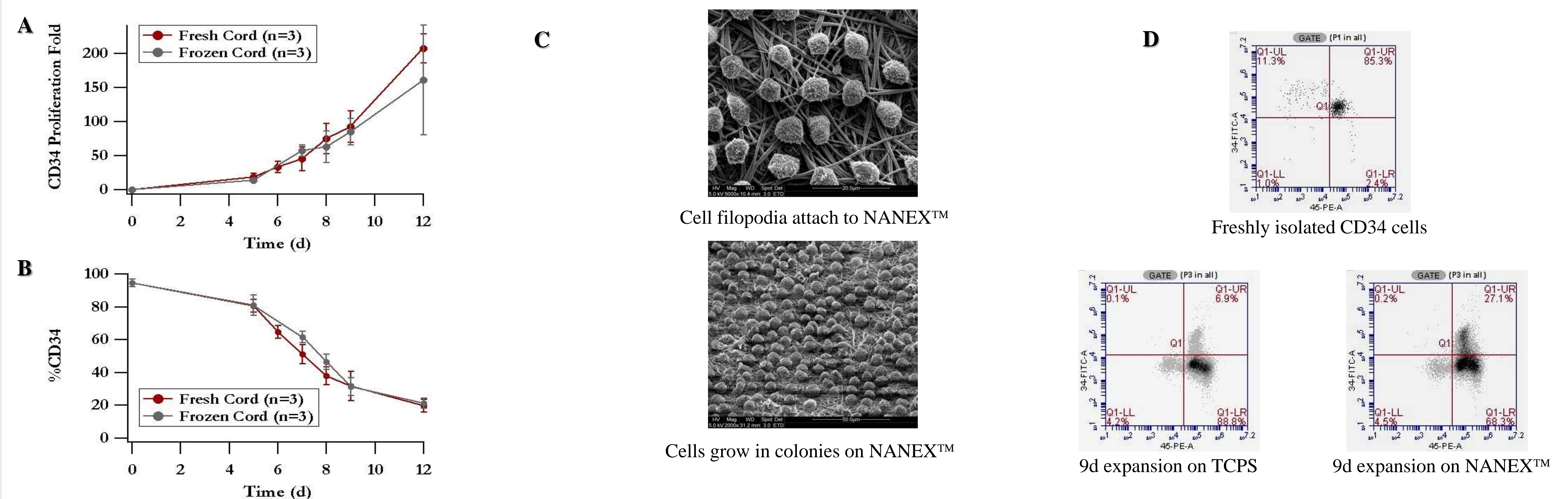
Fresh cord blood units (<24 hrs old) were obtained through the Hematopoietic Stem Cell Core Facility at Case Western Reserve University. Cord blood mononuclear cells were obtained via density gradient centrifugation over Ficoll-Paque™. CD34+ cells were isolated using an autoMACS® Pro Separator (Miltenyi Biotec) after immunomagnetic labeling with CD34 microbeads. CD34+ cells were either seeded onto substrates directly or cryopreserved, thawed, and then seeded. All cell culture was carried out using Arteriocyte's serum-free medium and growth factor cocktail. Cells were seeded at a density of 1,000 cells/mL.

Cell Harvesting and Analysis:

After the culture period, cells were harvested using enzyme-free cell dissociation buffer, counted, and immunostained for flow cytometry analysis. Cell counting was performed using a C6 Flow Cytometer (Accuri Cytometers) by adding Sphero™ Accucount Particles (Spherotech Inc.) to each sample. Analysis of surface marker expression was also carried out on the C6 Cytometer after immunostaining the cells with CD34-FITC and CD45-PE antibodies (Miltenyi Biotec).

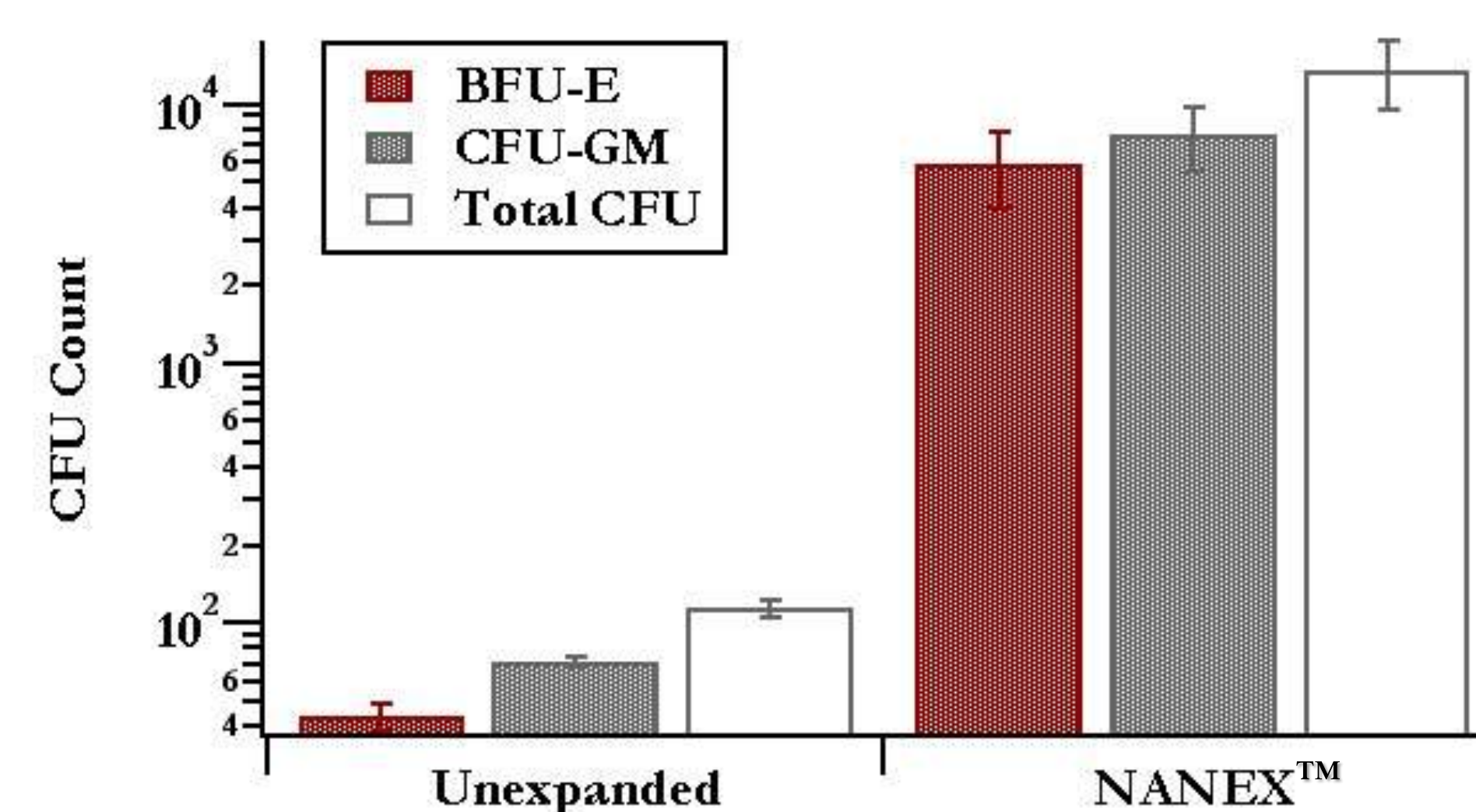
RESULTS

Freshly isolated and cryopreserved UCB CD34 cells proliferate extensively on NANEX™ substrate



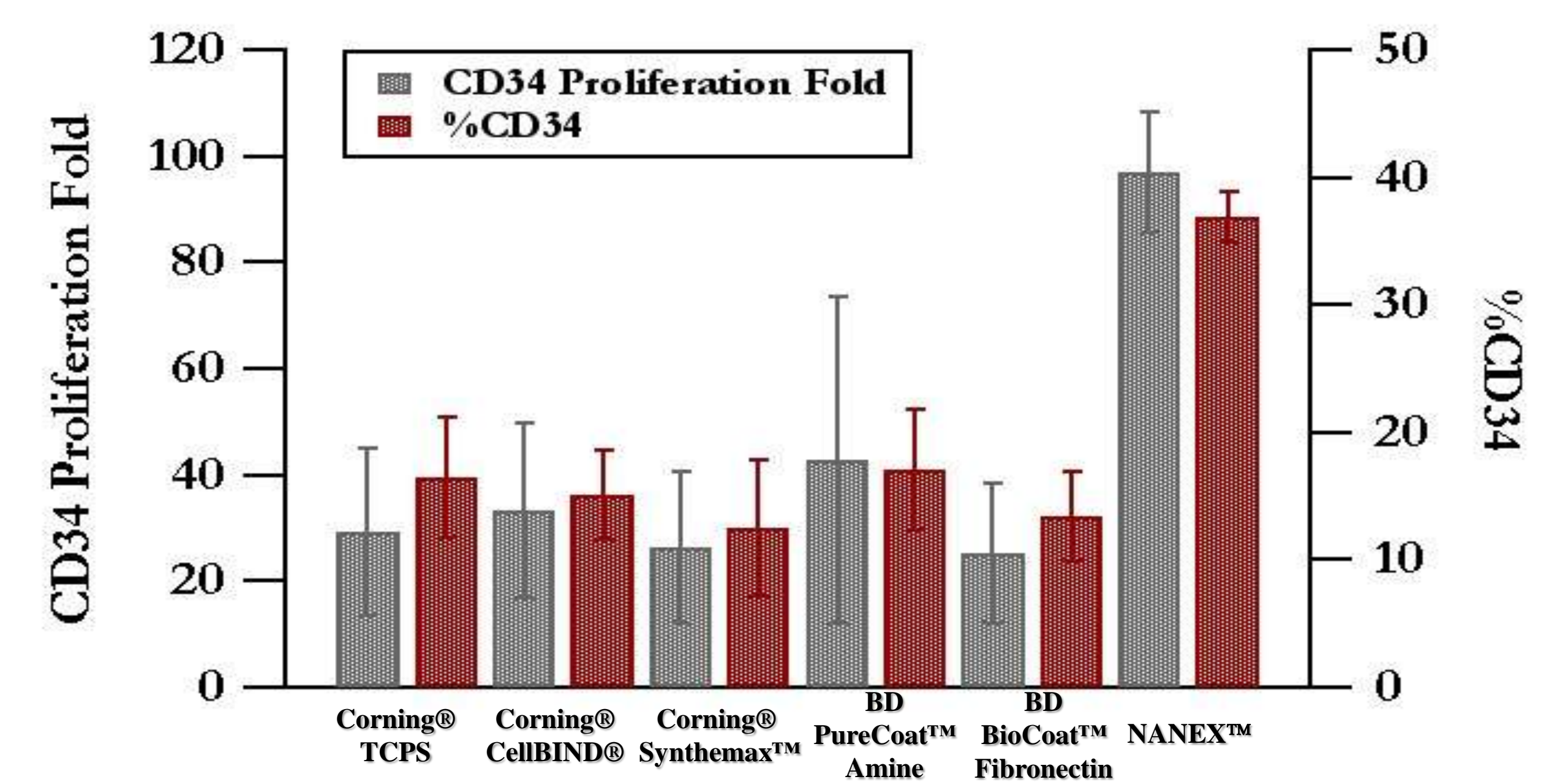
(A) CD34 proliferation fold as a function of time in culture for freshly isolated and cryopreserved UCB-derived cells CD34 cultured on NANEX™ substrates. Results are the average of three cords. (B) Percentage of freshly isolated and cryopreserved UCB-derived CD34 cells maintaining CD34 expression as a function of time in culture on NANEX™ substrates. Results are the average of three cords. (C) Scanning electron micrographs of UCB-derived CD34 cells cultured on NANEX™ scaffold at 2000X and 5000X magnification. Higher magnification image shows cell filopodia attaching to nanofibers. (D) Representative flow cytometry dot plots showing CD34 and CD45 expression for freshly isolated UCB-derived CD34 cells before and after 9-day culture on NANEX™ substrate or standard tissue culture polystyrene (TCPS, control).

NANEX™ culture leads to significant expansion of colony forming unit cells



Burst forming unit-erythroid (BFU-E), colony forming unit-granulocyte-macrophage (CFU-GM), and total CFU counts after 14 days of culture in MethoCult® H4435 medium (StemCell Technologies) for freshly isolated UCB CD34 cells (unexpanded) and for cells cultured for 8 days on NANEX™ substrate. Results are the average of two cords. Data is normalized to 600 unexpanded CD34 cells (initial seeding density per well).

NANEX™ demonstrates superior performance over other substrates



CD34 proliferation fold and CD34 percentage for freshly isolated UCB-derived cells cultured for 8 days on NANEX™ and other substrates. Results are the average of two cords. TCPS = tissue culture polystyrene.

CONCLUSIONS

- UCB-derived CD34 cells cultured on NANEX™ show enhanced proliferation and maintenance of the CD34 phenotype compared to other commercially available substrates.
- The NANEX™ substrate works equally well with both freshly isolated and cryopreserved UCB CD34 cells.
- Culturing UCB CD34 cells on NANEX™ leads to a significant increase in colony forming unit (CFU) cells, demonstrating its ability to expand progenitor cells.
- NANEX™ technology provides a robust *ex vivo* expansion of UCB-derived HSCs/HPCs and offers great promise for increasing the use of cord blood as an HSC source (the development of the NANEX™ platform for use with other cell types is currently being investigated).

ACKNOWLEDGEMENTS

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