Corning® PureCoat™ Surfaces Support Growth, Expansion, and Differentiation of Stem Cells

Jeff Partridge, M.S., Kerry E. Thompson, B.S., and Elizabeth J. Abraham, Ph.D.
Corning Incorporated, Tewksbury, MA, USA

Application Note

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Introduction

The promise of stem cells lies in their distinctive ability to differentiate along a multitude of cell lineages, thus considerable efforts are focused on identifying biomaterials that promote stem cell expansion and/or differentiation. Here, we report that Corning PureCoat amine, a chemically defined, animal-free, cell culture surface, supports increased growth of human bone-marrow-derived mesenchymal stem cells (hMSC) and human adipose-derived stem cells (hASC) culture over standard tissue culture-treated (TC-treated) vessels. When cultured on Corning PureCoat amine, these cell types grow and reach cell confluence faster than they do on a standard TC-treated surface while retaining established surface marker profiles that are characteristic of these cells. Both hASC and hMSC expanded on Corning PureCoat amine for multiple passages can be induced to differentiate into osteogenic or adipogenic lineages on TC-treated, Corning PureCoat amine, and Corning PureCoat carboxyl surfaces. This application note will focus on growth and differentiation of hMSC and hASC on Corning PureCoat surfaces.

Materials and Methods

Human Mesenchymal Stem Cell Culture

Bone-marrow-derived hMSC were purchased from Lonza. Cryopreserved cells were thawed and immediately seeded onto 6-well TC-treated or Corning PureCoat amine plates at 6,000 cells/cm^2 in 2 ml of growth medium (MSCGM™, Lonza) per well. After 4 days of growth, these cells (passage 2 or P2) were trypsinized and split at a 1:2 ratio. Cells were grown for an additional 3 days, re-passaged (P3) and split at a 1:5 ratio for two more successive passages (P4 and P5) each grown for approximately 4 days. Cells were subcultured using Clonetics® Trypsin-EDTA (Lonza) according to vendor recommendations. An aliquot of the cell suspension was removed to count using the Vi-CELL® automated cell counter.

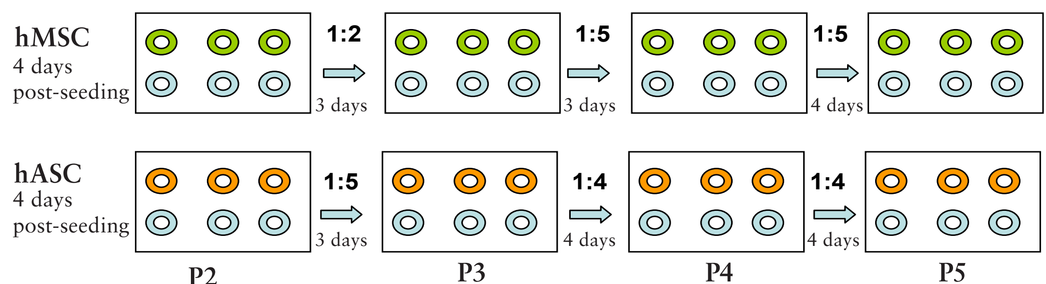
Cell confluence measurements
IncuCyte™ Plus (Essen Instruments) was utilized to collect phase contrast images over time. This program was used to non-invasively assess live cell cultures and measured cell confluence as a quantitative metric. Cell confluence measurements were taken from 9 regions within each well and values were averaged to calculate mean confluence/well.

Differentiation studies

Adipogenic lineage
hMSCs (P4 and P5) that were cultured on 6-well TC-treated or Corning PureCoat amine plates. Cells were trypsinized and seeded at 3,750 cells/ cm^2 onto 24- or 6-well TC-treated and Corning PureCoat carboxyl plates at 37°C, in a humidified 5% CO_2 atmosphere and allowed to grow in MSCGM™ medium. Upon reaching 100% confluence, cell monolayers were subjected to three cycles of induction according to the protocol recommended by Lonza. Each cycle consists of feeding hMSCs with supplemented Adipogenesis Induction media for 3 days followed by 1-2 days in Maintenance media (Lonza). Control wells were re-fed with Maintenance media for the duration of the differentiation protocol. After the third induction cycle, cultures were washed with 1X PBS, fixed with 4% paraformaldehyde and stained with Oil-Red-O.

Osteogenic lineage
hMSCs (P3) that were cultured on 6-well Corning PureCoat amine plates were trypsinized and seeded at 3,000 cells/cm^2 into 6-well TC-treated and Corning PureCoat amine or carboxyl plates and cultured at 37°C in a humidified 5% CO_2 atmosphere. The next day, cells that were induced to differentiate were fed Osteogenesis Induction Medium (Lonza) whereas the non-induced hMSCs were fed with MSCGM medium. These cultures were re-fed every 3-4 days and after 2-3 weeks of differentiation, cultures were washed with 1X PBS, fixed with 4% paraformaldehyde and stained with Alizarin Red. Calcium deposits stained red in induced cells whereas control wells were devoid of color.

Experimental Design



Shown above is an example of the design for these experiments. 2-3 wells were pooled to obtain one sample and multiple plates were assessed for each experiment (n=4-6 samples/surface).

Flow Cytometery Analysis (FACS)
Cells (P4) grown on 6-well Corning PureCoat amine or TC-treated plates were prepared for FACS. After removal from growth surfaces, cells were resuspended in washing buffer consisting of DPBS with 5% serum, and stained with antibodies for 1h at 4°C. A minimum of 10,000 events was recorded for each analysis, using a BD FACSCalibur™ flow cytometer. To exclude debris and dead cells, appropriate gating was performed using forward and side scatter. The reagents used were as follows; CD34-FITC, CD44-FITC, CD45-FITC, CD90-FITC, and CD29-APC (BD Cat. Nos. 555821, 555478, 555482, 555595, 559883, respectively). Apart from using an Isotype control, each antibody was also compared with an irrelevant negative control.

Human Adipose-derived Stem Cells
hASCs were purchased from Invitrogen™. Cryopreserved cells were thawed and immediately seeded onto 6-well TC-treated or Corning PureCoat amine surface in serum-containing MSCGM™ media (Lonza) at approximately 6,000 cells/cm^2. After 4 days of growth, P2 cells were trypsinized and split at a 1:5 ratio. Cells were grown for 4 days, re-passaged (P3) and split at a 1:4 ratio for two more successive passages (P4 and P5) each grown for approximately 4 days. Cells were washed with 1X DPBS and subcultured using Clonetics® Trypsin-EDTA (Lonza). The dissociation reagent was neutralized with MSCGM and an aliquot of the cell suspension was removed to count using the Vi-CELL® automated cell counter.

Results and Discussion

Improved hMSCs cell yields on Corning PureCoat amine surface
The Corning PureCoat amine surface supports the growth of hMSCs as shown in Figure 1. Multiple lots (2 donors) of these cells have been successfully cultured on Corning PureCoat amine with distinct advantages over TC-treated surface. An increased number of cells are obtained more rapidly on Corning PureCoat amine as compared to a TC-treated surface with increases ranging from 25% to 140% across several passages (P3-P6). This growth advantage starts slow but gains momentum by P3 and is continued through P6. Shown are calculated hMSC yields from multiple donors. We observed donor to donor variations in cell growth during our assessment. Cell growth as measured by total cell yield, peaked at P4 and slowed down by P6 in Donor 2 which was different from the response with Donor 1.

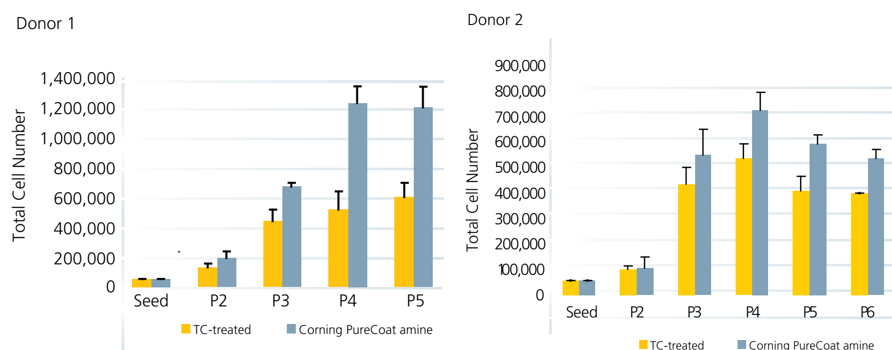
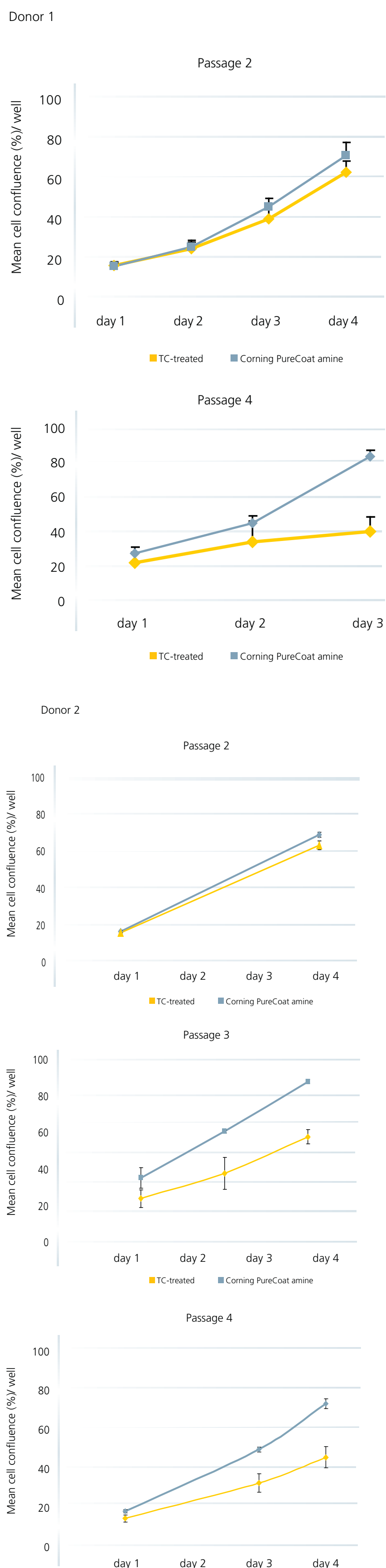


Figure 1. Cryopreserved hMSCs (passage 2) were thawed and immediately seeded onto 6-well TC-treated (TC) or Corning PureCoat amine plates in serum-containing MSCGM media (Lonza) at ~6,000 cells/cm^2. Shown are calculated hMSC yields where total number of cells collected was multiplied by the dilution or split ratio to yield total number of cells for that passage. An increased number of cells are obtained more rapidly on Corning PureCoat amine as compared to a TC-treated surface with increases ranging from 25% to 140% across several passages (P3-P6). This growth advantage starts slow but gains momentum by P3 and is continued through P6.



More rapid hMSC cell growth on Corning PureCoat amine

Figure 2 depicts growth kinetic plots using the IncuCyte™ and calculates surface area occupied by cells, which is then expressed as percent cell confluence. Growth kinetics on Corning PureCoat amine show smaller gains initially (P2), but increase steadily by P3. These gains continue to be observed through later passages (up to P6) when compared to TC-treated surface. Cells grow more rapidly on Corning PureCoat amine, thus obtaining greater cell confluence in a shorter period of time when compared to TC-treated. Cells were split when either surface reached >70-80% cell confluence, and a constant split ratio was maintained on Corning PureCoat amine and TC-treated surfaces as depicted in the Experimental Design (see Materials and Methods). Figure 3 depicts images of hMSC and hASC cultures grown for multiple passages on the Corning PureCoat amine surface immediately prior to sub-culturing.

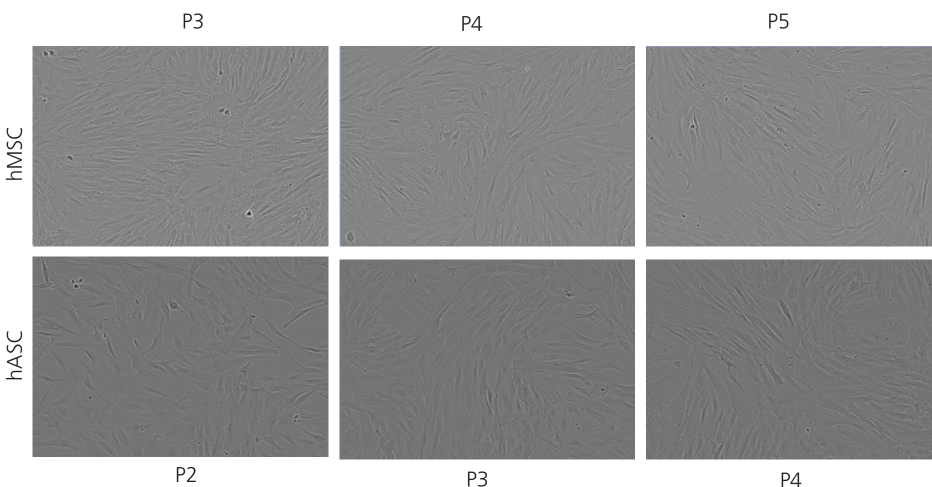


Figure 3: Microscopic images of hMSCs and hASCs grown for multiple passages on Corning PureCoat amine immediately prior to sub-culturing.

Figure 2: Evaluation of cell growth kinetics of hMSCs on 6-well TC-treated and Corning PureCoat amine surfaces. hMSC cell growth was monitored over several passages using the IncuCyte™ from Essen Instruments. Shown are cell confluence growth curves for both donors over time across multiple passages. As shown, hMSCs grown on Corning PureCoat amine reach higher cell confluency more rapidly than those cells grown on the TC-treated surface after the post-thaw lag-phase.

hMSCs cultured on Corning PureCoat amine are phenotypically similar to those cultured on TC-treated surface and retain differentiation potential

hMSCs grown on Corning PureCoat amine express surface antigens similar to those cells cultured on the TC-treated surface. Namely, they express CD29, CD90, CD44 and are devoid of CD34 and CD45 antigens as shown in Figure 4. Another hallmark of hMSCs is their ability to differentiate into specific tissue lineages. Here we show that hMSCs grown on Corning PureCoat amine can differentiate into osteogenic and adipogenic lineages. hMSCs expanded on Corning PureCoat amine for multiple passages were induced to differentiate on three different surfaces: TC-treated, Corning PureCoat amine, and Corning PureCoat carboxyl. We report that hMSCs cultured on Corning PureCoat were shown to differentiate into adipogenic lineage when induced to differentiate on TC-treated or Corning PureCoat carboxyl surfaces (Figure 5). In addition, TC-treated and both Corning PureCoat amine and carboxyl surfaces were shown to support differentiation of the Corning PureCoat amine-expanded hMSCs into an osteogenic lineage (Figure 6). Upon differentiation, the hMSC cell morphology changes, whereas non-induced cells remain undifferentiated. For positive controls, hMSCs expanded on TC-treated surface were induced to differentiate into osteogenic or adipogenic lineages.

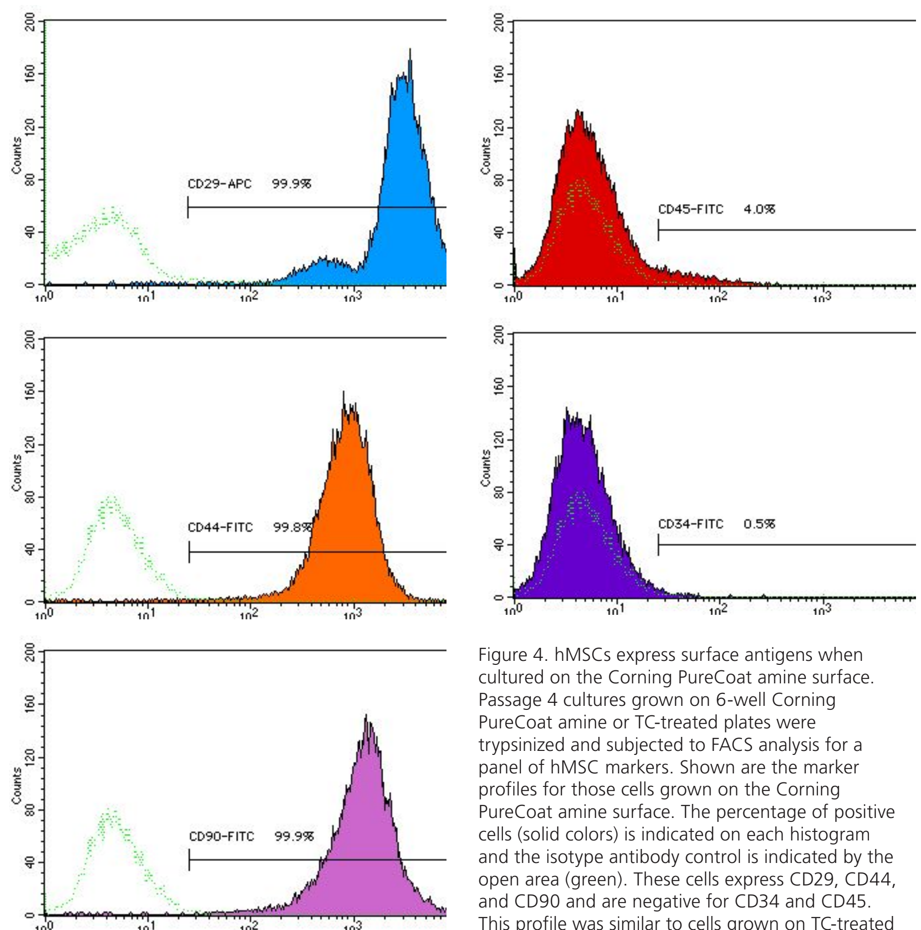


Figure 4. hMSCs express surface antigens when cultured on the Corning PureCoat amine surface. Passage 4 cultures grown on 6-well Corning PureCoat amine or TC-treated plates were trypsinized and subjected to FACS analysis for a panel of hMSC markers. Shown are the marker profiles for those cells grown on the Corning PureCoat amine surface. The percentage of positive cells (solid colors) is indicated on each histogram and the isotype antibody control is indicated by the open area (green). These cells express CD29, CD44, and CD90 and are negative for CD34 and CD45. This profile was similar to cells grown on TC-treated (data not shown).

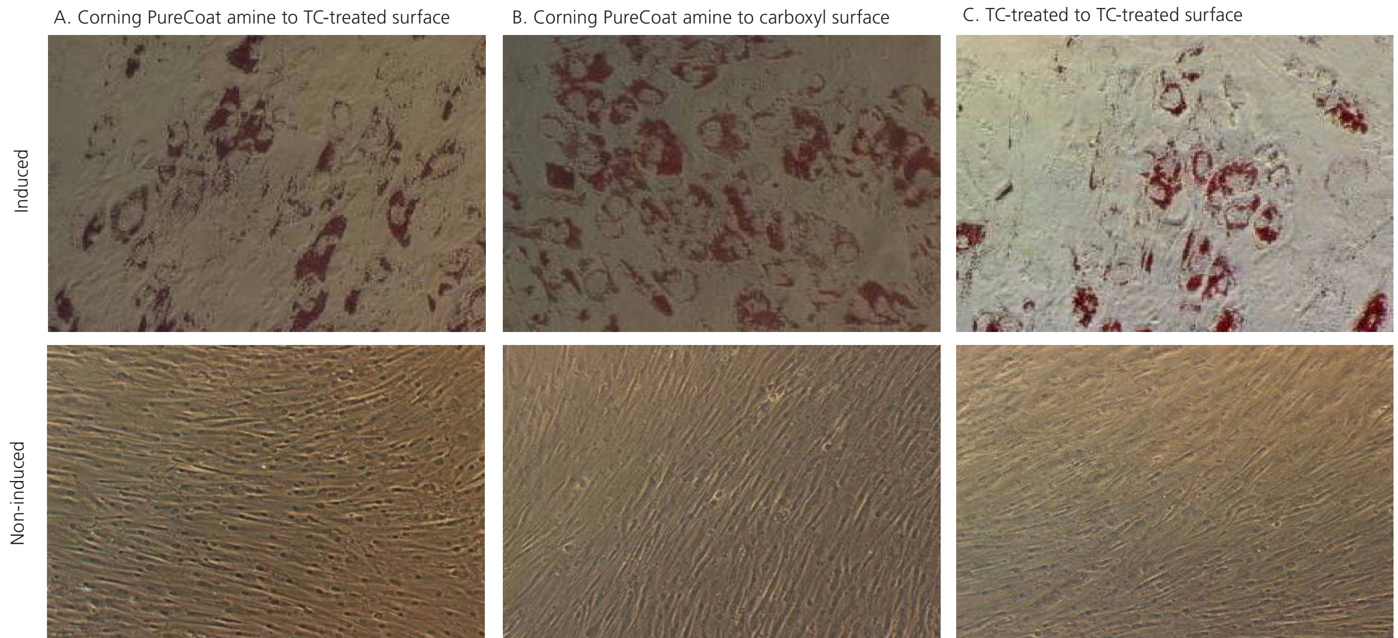


Figure 5. Adipogenic differentiation potential of hMSCs cultured on Corning PureCoat amine surface immediately prior to sub-culturing. hMSCs (passage 4) that were cultured on 6-well Corning PureCoat amine plates were trypsinized and seeded into 6- or 24-well TC-treated (panel A) and Corning PureCoat carboxyl (panel B) plates. Cells were then induced to differentiate into adipogenic lineage. Post-differentiation, cultures were washed with 1X PBS, fixed with 4% paraformaldehyde and stained with Oil-Red-O. Intracellular lipid vacuoles were observed in induced cells and were absent in non-induced cultures. TC-treated expanded hMSCs served as the positive control (panel C).

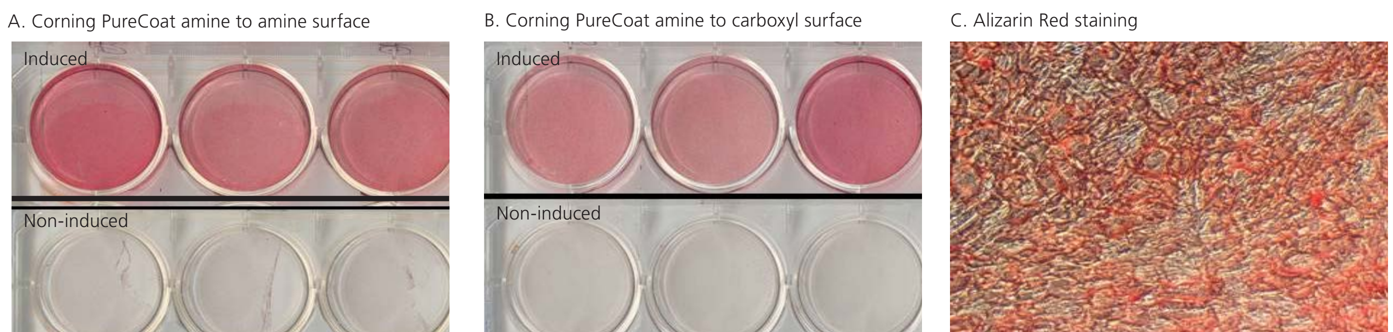


Figure 6: Osteogenic differentiation potential of hMSCs grown on the Corning PureCoat amine surface. hMSCs (P3) that were cultured on 6-well Corning PureCoat amine plates were trypsinized and seeded onto 6-well TC-treated (data not shown) and Corning PureCoat amine (panel A) or carboxyl (panel B) plates. Cells were then induced to differentiate into osteogenic lineage. Post-differentiation, cultures were washed with 1X PBS, fixed with 4% paraformaldehyde and stained with Alizarin Red. Both scanned images of the plates (panel A and B) as well as a representative microscopic image post Alizarin red staining of an induced culture is shown in panel C. Calcium deposits stained red in induced cells whereas control wells were devoid of color. Both Corning PureCoat surfaces supported osteogenic differentiation.

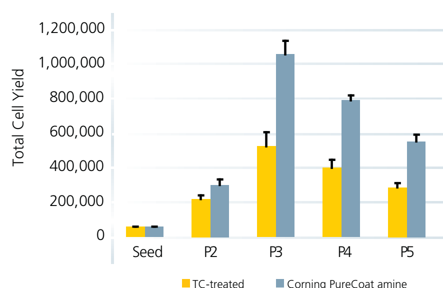


Figure 7: Increased growth of hASCs on Corning PureCoat amine. Cryopreserved hASCs (P2) were thawed and immediately seeded onto 6-well TC-treated or Corning PureCoat amine plates in serum-containing MSCGM media (Lonza) at ~6,000 cells/cm^2. Shown are calculated hASC yields where total number of cells collected was multiplied by the dilution or split ratio to yield total number of cells for that passage. Cells were passaged between 70-80% growth confluency as measured by Incucyte™ and the split ratio was kept constant across both TC-treated and Corning PureCoat amine surface. We observed greater total cell yield of hASCs cultured on Corning PureCoat amine and the increases in cell yield compounded with every passage.

Increased cell yields of human adipose-derived stem cells (hASCs) grown on Corning PureCoat amine

Here we report that the Corning PureCoat amine surface supports increased and more rapid growth of hASCs when compared to a TC-treated surface in medium containing serum. Increases in cell yield are maintained across several passages and peak around P3 (Figure 7). hASCs (P4) cultured on Corning PureCoat amine surface were also evaluated for their ability to differentiate into adipogenic lineage. We report that adipogenic differentiation was achieved when these cells were induced either on Corning PureCoat carboxyl or TC-treated surfaces (data not shown).

Conclusions

- 6-well formats of Corning PureCoat amine support enhanced growth of human bone marrow-derived mesenchymal stem cells and adipose-derived stem cells.
- hMSCs cultured on Corning PureCoat amine express surface markers characteristic of these cells.
- hMSCs and hASCs cultured on Corning PureCoat amine retain differentiation potential. Corning PureCoat amine-expanded hMSCs can be induced to differentiate into adipogenic and osteogenic lineages on TC-treated and Corning PureCoat carboxyl or amine surfaces.

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Corning Incorporated, One Riverfront Plaza, Corning, NY 14831-0001

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Corning Incorporated
Life Sciences

Corning BV
Fogostraat 12
1060 LJ Amsterdam
The Netherlands
Phone: +31 0 20 659 60 51
Fax: +31 20 659 76 73
cceurnl@corning.com
www.corning.com/lifesciences

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