Application Note

Expansion and differentiation of human mesenchymal stromal cells

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Introduction

Human mesenchymal stromal cells (hMSC) are candidates for clinical use because they are readily expanded in culture, have immuno-modulatory potential and can differentiate into the osteogenic, chondrogenic and adipogenic lineages. Their therapeutic potential is currently studied as part of clinical trials to treat diseases such as graft-versus-host disease[1] and osteoarthritis[2], as well as in the regeneration of cardiac muscle following myocardial infarcts[3]. Whether the requirements are for clinical or research use, obtaining a substantial number of cells can constitute a bottleneck for the investigator. hMSC display some plasticity in their culture conditions, but several investigators report a higher growth index and increased differentiation potential at lower seeding densities[4,5]. We here present a protocol enabling the clinician or researcher to rapidly expand a population of hMSCs on Thermo Scientific Nunclon Delta cell culture treated surface utilizing the potential of Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell Basal Medium, developed specifically for the optimal expansion and maintenance of undifferentiated hMSCs. A definitive test of multi-potency is a functional test. In consequence, we subjected the expanded hMSC to differentiation. The cells were differentated into osteoblast or adipocytes in Thermo Scientific Nunc 48 well multidishes.

Methods

Cultivation of hMSC. Human mesenchymal stromal cells (Lonza, USA) were maintained in α-MEM medium containing 10% FBS, 1% Penicillin/Streptomycin and 2mm UltraGlutamine, or AdvanceSTEM™ Mesenchymal Stem Cell Basal Medium supplemented with 10% AdvanceSTEM Growth Supplement.

In order to test the effect of cell disassociation on the growth and differentiation of the hMSCs.

Cell culture: hMSC was incubated at 37°C in a humidified atmosphere of 5% CO_2 in air using a Thermo Scientific Revco Ultima II Series CO_2 Incubator.

Cell counting: Cells were counted using an integrated automated fluorescence microscope (Nucleocounter, Chemometec, Denmark).

Growth curves: To develop the protocol, growth curves of hMSC cultivated in AdvanceSTEM and α-MEM growth media were established. hMSCs in passage 2 were seeded at 100, 350, 1000 and 4000 cells/cm^2 in Nunclon™ Delta treated T25 flasks. The cultures were placed in an IncuCyte Plus and incubated at 37°C in a humidified atmosphere of 5% CO_2. The IncuCyte Plus is an automated imaging platform, configured to fit inside a CO_2 incubator, and designed to provide kinetic, non-invasive live cell imaging by acquiring phase-contrast images of the cells at user-defined times and locations within the cultures. The primary metric of the instrument is culture confluence, that is, the fraction of the surface that is covered by cells. The cells were cultivated for 12 days with media change every 4 days.

Expansion protocol: A single cryovial with 5x10^5 cells was thawed (cells in passage 2), and the percentage of viable cells was established. The cells were divided and seeded in two Nunclon Delta treated TripleFlasks with a final seeding density of 350 cells/cm^2 with either AdvanceSTEM Mesenchymal Stem Cell Basal Medium or α-MEM growth media. The cells were cultivated for 8 days with media change on Days 3 and 7. The expanded cells were re-seeded in a Nunclon Delta treated TripleFlasks at seeding density of 350 cells/cm^2. The cells were cultivated for 8 days with media change on Day 4.

Differentiation protocol: After the expansion protocol cells were harvested with trypsin and re-seeded in AdvanceSTEM or α-MEM in 48 well multidishes at a density of 5000 cells/cm^2 for differentiation into osteoblasts and adipocytes. The cells were incubated for 48 hours at which time the media in the 48 well multidishes were changed to either AdvanceSTEM Adipogenic or osteogenic differentiation media with AdvanceSTEM Growth Supplement. The cells were incubated for 18 days with the media being changed every 4-5 days. The cells were assayed for differentiation using the following commercial kits: For Osteogenic differentiation, the OsteoImage PA-1501 kit was used. The kit measures specific staining of the hydroxyapatite portion of the bone-like nodules deposited by cells. For adipogenic differentation, the AdipoRed PT-7009 was used. The kit utilizes Nile Red to dye the intracellular lipid droplets formed inside the differentiating adipocytes. The differentiation cultures were assayed on Days 3, 7 and 18.

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The growth of hMSCs seeded at four different densities in two different media (α-MEM medium and AdvanceSTEM Mesenchymal Stem Cell Basal Medium) was investigated. hMSCs were cultured for 12 days and the growth was followed in the incubator using the IncuCyte Imager. For the first 50 hours, at 1000 and 4000 cells/cm^2, the growth of hMSCs in α-MEM medium and AdvanceSTEM Mesenchymal Stem Cell Basal Medium displayed a similar growth pattern (Fig. 1A), but then the cultures diverged. The growth rate of hMSC in α-MEM medium declined and reached a plateau at approximately 80% culture confluence after approximately 100 hours for cultures seeded at 4000 cells/cm^2 and 150 hours for cultures seeded at 1000 cells/cm^2. The growth of cultures of hMSCs seeded at 1000 and 4000 cells/cm^2 in AdvanceSTEM Mesenchymal Stem Cell Basal Medium continued to grow past 80% culture confluence and were able to reach 96-99% confluence.

At 350 cells/cm^2, the cultures seeded in α-MEM medium plateaued at around 75% confluence after 180 hours and cells seeded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium reached the same level after approximately 250 hours.

At the lowest seeding density of 100 cells/cm^2, hMSC cultured in α-MEM medium reached a plateau of 67% at approximately 235 hours. The cells seeded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium were still in a slow exponential growth phase at the experiments end at 300 hours with a confluence of approximately 40%. The purpose of our protocol is to expand a relatively low number of cells; we thus chose the relatively low seeding density, which displayed good exponential growth, of 350 cells/cm^2. The morphology of hMSCs cultured in the two media was similar (Figs. 1B and 1C).

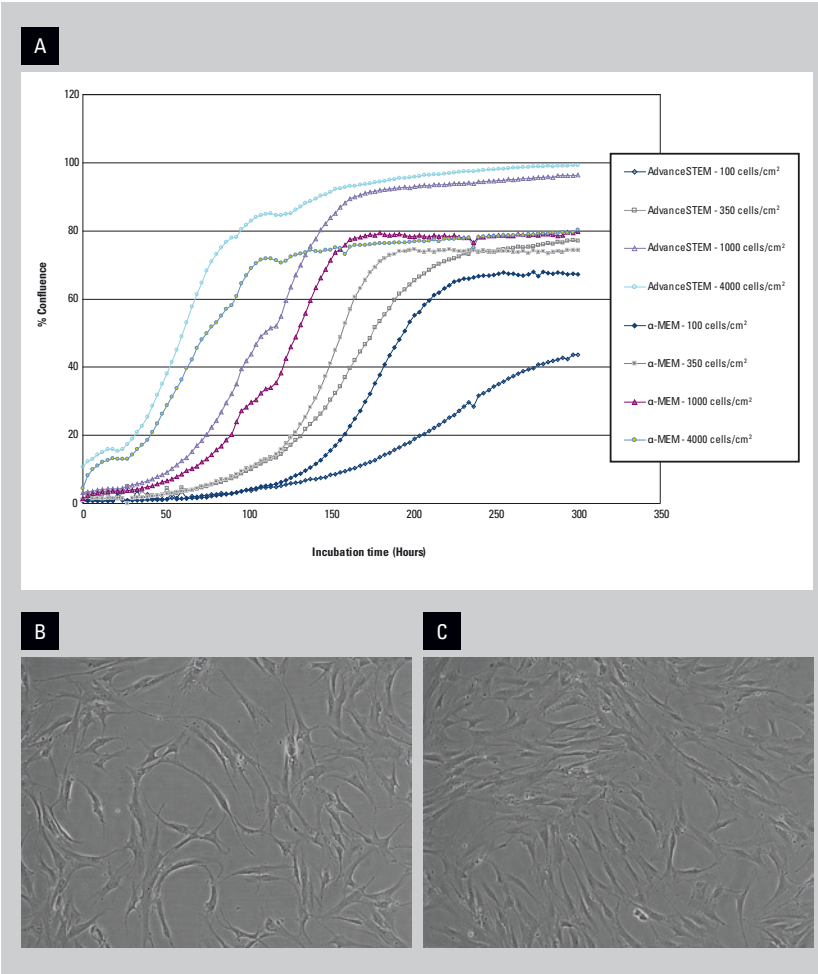


Fig. 1.

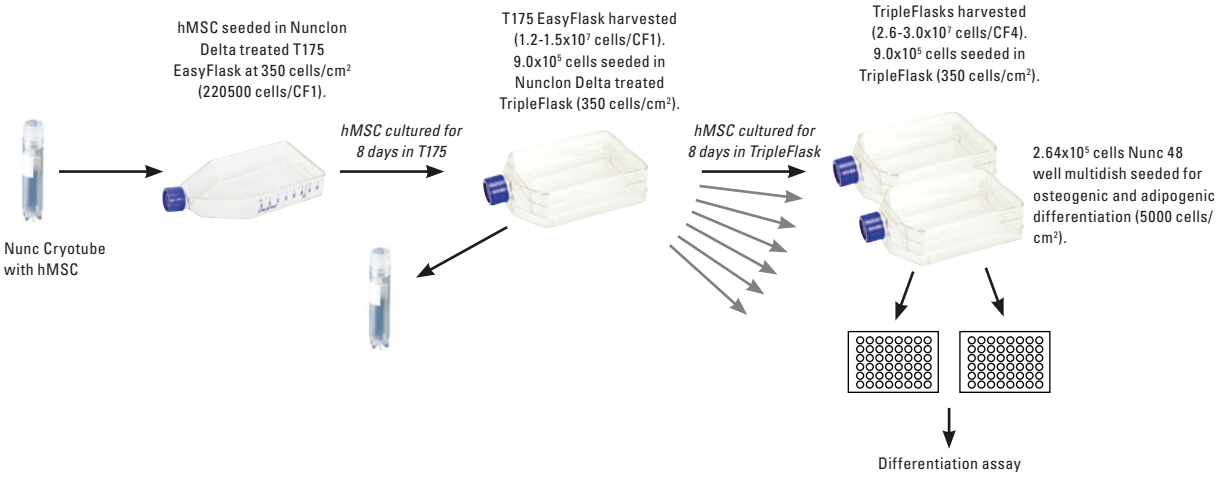
A: Growth of hMSC in T25 flasks with Nunclon Delta surface in α-MEM medium or AdvanceSTEM Mesenchymal Stem Cell Basal Medium. Cells were seeded at four different densities: 100-350-1000-4000 cells/cm^2. Cultures were incubated for 12 days under standard culture conditions (5% CO_2 with media change every fourth day. Culture confluence was measured using automated microscopy in the incubator (IncuCyte Imager) every three hours. Each data point represents the mean of 50 measurements in one flask.

B: hMSC morphology in AdvanceSTEM Mesenchymal Stem Cell Basal Medium after 7 days of incubation. Seeding density: 350 cells/cm^2.

C: hMSC morpholgy in α-MEM medium after 7 days of incubation. Seeding density: 350 cells/cm^2.

Nunclon Delta treated TripleFlasks an effective format for the cultivation of hMSC

The growth of hMSC in α-MEM medium and AdvanceSTEM Mesenchymal Stem Cell Basal Medium TripleFlasks using our in-house developed protocol was effective in generating a large population of hMSCs for either differentiation or cryogenic storage.



hMSCs maintain their multi-potency after large scale expansion in Nunclon Delta treated TripleFlasks

In order to verify that the cells had maintained their ability to differentiate, the cells expanded in TripleFlasks, in both types of growth media, using our in-house protocol, was differentiated into osteoblasts and adipocytes in 48 well multidish at a density of 5000 cells/cm². The differentiation was induced using either osteogenic or adipogenic differentiation media. The differentiation was monitored at Days 3, 7, and 18 using commercial kits.

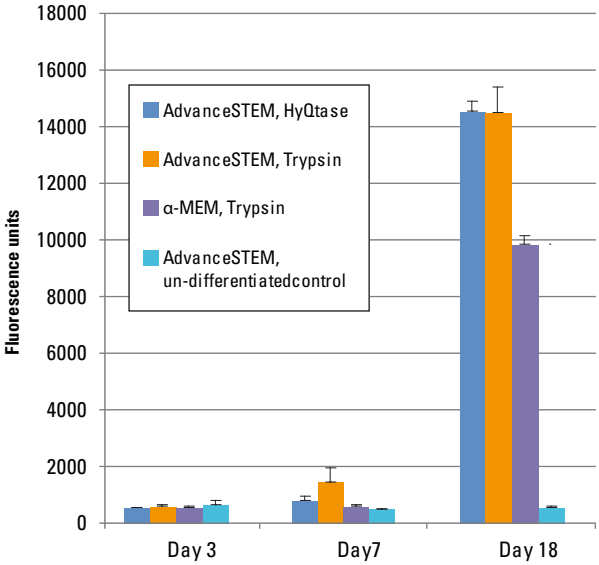


Fig. 2. OsteoImage data from hMSCs osteogenically differentiated in 48 well multidish and assayed at day 3, 7, and 18 post-change to differentiation media. Data displayed as relative fluorescence units.

Impact of AdvanceSTEM Mesenchymal Stem Cell Basal Medium on differentiation

Marked differences between hMSCs expanded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium and hMSCs expanded in α-MEM medium was observed during differentiation. hMSCs expanded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium displayed a 59% higher signal using the OsteoImage assay compared to cells expanded in α-MEM medium (Fig. 2). Regarding adipogenic differentiation, hMSCs expanded in α-MEM medium were unable to differentiate into adipocytes displaying baseline signals in the AdipoRed assay. In contrast, hMSCs expanded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium differentiated successfully into adipocytes (Fig. 3).

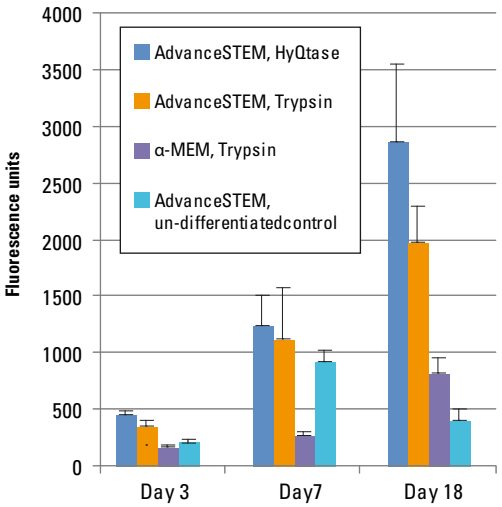
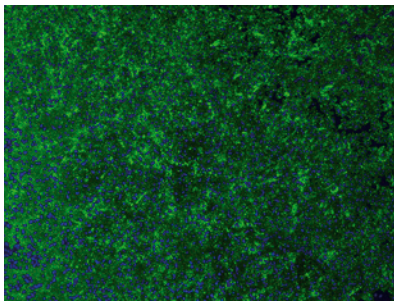
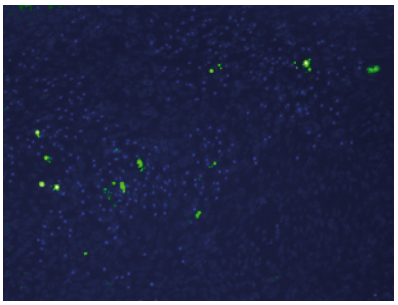


Fig. 3. AdipoRed data from hMSCs differentiated into adipocytes in 48 well multidish. The cells were assayed at Days 3, 7, and 18 post-change to differentiation media. Data displayed as relative fluorescence units.

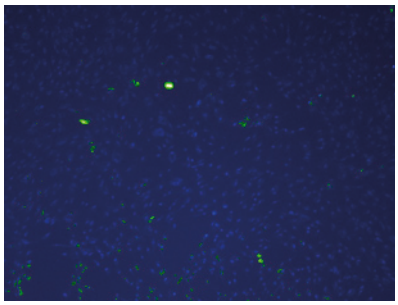
Fig. 4.



Composite OsteoImage/Hoechst stained hMSCs differentiating into osteoblasts. The cells are photographed at day 18 post induction. The hMSC were expanded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium and passaged using trypsin.



Composite AdipoRed/Hoechst stained hMSCs differentiating into adipocytes. The cells are photographed at day 18 post induction. The hMSC were expanded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium and passaged using trypsin.



Composite AdipoRed/Hoechst stained hMSCs differentiating into adipocytes. The cells are photographed at day 18 post induction. The hMSC were expanded in α-MEM medium and passaged using trypsin.

Results

The lowest seeding concentration displaying good exponential growth was 350 cells/cm^2. In consequence a seeding density of 350 cells/cm^2) were chosen for the expansion protocol. In our experiment we expanded the original hMSC population.

Differentiation into adipocytes and osteoblasts were possible with cells expanded in HyClone AdvanceSTEM Mesenchymal Stem Cell Basal Medium, but only osteogenic differentiation, at a lower yield, was possible with cells expanded in α-MEM medium.

Conclusions

- Nunclon Delta treated surfaces including TripleFlasks are an effective format for the easy and rapid expansion of hMSC
- Expansion of hMSC on Nunclon Delta using AdvanceSTEM Mesenchymal Stem Cell Basal Medium does not compromise the osteogenic and adipogenic potential of the hMSCs
- A substantially higher yield of osteoblast is achieved when cells are expanded in AdvanceSTEM Mesenchymal Stem Cell Basal Medium compared to α-MEM medium.

References

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