Collagenase Type I for Enzymatic Passaging of Human Embryonic Stem Cells in HEScGRO™ Medium

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Abstract

Human embryonic stem cells (hESCs) cultured in HEScGRO, a proprietary serum-free and animal-component-free medium from Millipore[1], cannot be passaged with collagenase type IV, a commonly-used enzyme, as it causes cells to rapidly differentiate. However, as shown here, hESCs cultured in HEScGRO medium can easily be passaged with collagenase type I. This new offering from Millipore, along with a related product, Accumax[2], provides users with robust options for the enzymatic passaging of hESCs.

Introduction

Human embryonic stem cells are commonly passaged by one of two methods. Manual passaging is gentler on the cells but is time-consuming and tedious. Enzymatic passaging, on the other hand, allows for higher throughput but is associated with higher cell mortality and possible karyotypic instability[3]. Until now, hESCs cultured in HEScGRO medium could only be passaged manually, as enzymatic dissociation with trypsin or collagenase type IV causes the cells to differentiate within two to three passages. However, two new methods are now available for use with HEScGRO medium: Accumax solution, as described in the accompanying article in this volume[2], and as we show here, collagenase type I.

Methods

H9 (WA09) hESCs were maintained on a layer of Detroit 551 human fetal skin fibroblasts (ATCC Catalogue No. CCL-110) seeded at a density of 60,000 fibroblasts/cm^2 on tissue culture plates coated with 0.1% gelatin (Millipore). hESCs were maintained in HEScGRO medium human ES cell culture medium; media was replaced every 1-2 days. Cultures were passaged every 7 days.

Collagenase type I (Millipore) was used as a 4 mg/mL working solution. To prepare the working solution, 400 mg collagenase type I powder was dissolved in 100 mL DMEM/F12 (Millipore) and filter-sterilized with a 0.22 µm Stericup®-GP filter unit; the solution was then aliquoted and stored at -20 °C until needed.

To passage hESCs using collagenase type I, the HEScGRO culture medium was removed from cells growing as described above. Cells were overlaid with approximately 0.05-0.1 mL/cm^2 of the collagenase type I working solution and placed at 37 °C in a humidified cell culture incubator. After 10 minutes, the cultures were checked for signs of curling up at the edges of colonies (similar to what is seen during treatment with collagenase type IV in non-animal-free culture media); cells were then put back at 37 °C for another 5 minutes (i.e., 15 minutes total) before proceeding.

To remove cells from the culture surface, the surface was washed with HEScGRO Basal Medium at a volume ratio of 3:1 (HEScGRO Basal Medium:collagenase type I) with a serological pipette. Cells were removed by repeated washing with the same volume of HEScGRO Basal Medium; cells were never scraped off the culture surface, as this increased differentiation upon replating the cells. Cells were next spun at 75xg for 5 minutes, washed with HEScGRO Basal Medium and spun again. The pellet was then resuspended with HEScGRO Medium (for human ES cell culture) and replated on a fresh feeder layer of Detroit 551 human fibroblasts at an appropriate split ratio (according to the density of the original culture; usually 1:3 to 1:6).

Results

hESCs were cultured in HEScGRO hESC culture medium and were passaged for 12 successive passages using collagenase type I to dissociate the cells. During actual dissociation, collagenase type I causes the edges of hESC colonies to lift or round up on the plate surface, but does not appear to cause the colonies to fragment (not shown). However, after the 15 minute treatment at 37 °C, cells generally wash off the plate surface as pieces of colonies rather than whole colonies, and repeated pipetting breaks up these pieces further. This is in contrast to treatment with collagenase type IV as with non-animal-free hESC media, where the cells need to be scraped from the plate surface even after prolonged treatment with collagenase type IV.

hESCs passaged with collagenase type I maintained pluripotent characteristics typical of cells cultured in HEScGRO medium on a human fibroblast feeder layer. Colonies were irregularly shaped, with a sharp boundary at colony edges (Figure 1). Cells maintained a high nuclear-to-cytoplasmic ratio, and the nucleoli were often visible (not shown). After 12 passages with collagenase type I, hESCs expressed pluripotent markers (Figure 2) including alkaline phosphatase (Figure 2A) and TRA-1-60 (Figure 2B). Finally, these cells maintained a normal karyotype (not shown).

Discussion

This work demonstrates the utility of collagenase type I for the enzymatic passaging of human ES cells when culturing with HEScGRO hESC culture medium. As with other enzymatic techniques used in non-animal-free hESC media, use of collagenase type I saves time and effort compared to manual passaging. Furthermore, cells passaged multiple times with collagenase type I express pluripotency markers and maintain their normal karyotype.

HEScGRO medium users now have two ways to enzymatically passage hESCs: Accumax solution (see reference 2) and collagenase type I. The main differences between these two is that while Accumax solution more rapidly dissociates hESC colonies (5 minutes at room temperature), dissociation with collagenase type I results in larger colony fragments and therefore larger colonies after replating.

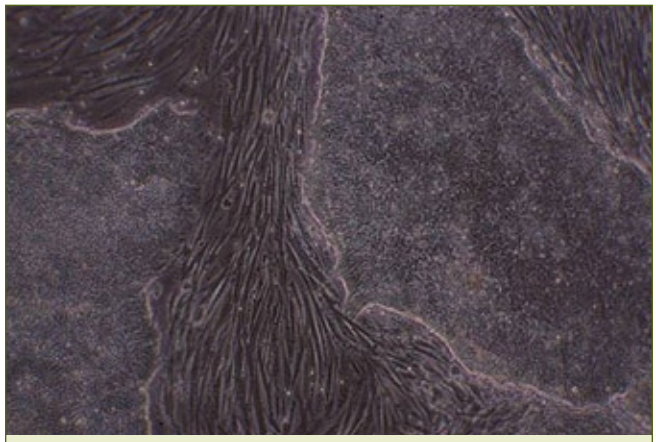

Figure 1. H9 (WA09) human ES cells cultured in HEScGRO serum- and animal-free medium, and passaged with collagenase I for 5 passages. Note the well-defined boundaries of human ES cell colonies surrounded by the human fibroblast feeder layer.

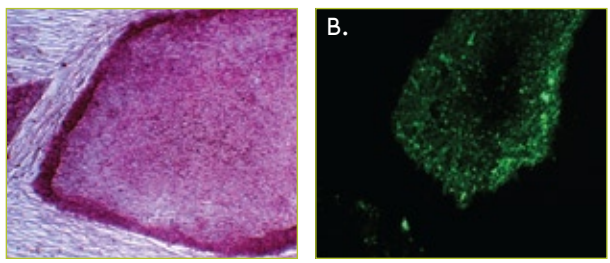

Figure 2. H9 (WA09) human ES cells cultured in HEScGRO medium express pluripotent markers after being passaged 12 times with collagenase I. (A) These cells express alkaline phosphatase, as shown via Alkaline Phosphatase Detection Kit. (B). These cells also express TRA-1-60, as revealed by indirect immunofluorescence with a mouse anti-TRA-1-60 antibody.

References

1. Singer M, *et al.* HEScGRO xeno-free medium for human embryonic stem cell culture. *Cellutions* **1**, 3-5 (2007).
2. Emre N, Mondeh R and Singer M. The use of Accumax solution for enzymatic passaging of human embryonic stem cells cultured in HEScGRO medium. *Cellutions* **1**, 16-18 (2007).
3. Hanson C, Caisander G. Human embryonic stem cells and chromosome stability. *APMIS* **113**: 751–5 (2005).

Millipore Products

Description	Qty/Pk	Catalogue No.
Collagenase Type I **Available July 2008**	250 mg	SCR103
HEScGRO Medium	5 x 100 mL	SCM020
HEScGRO Basal Medium	5 x 100 mL	SCM021
Accumax Solution	100 mL	SCR006
Alkaline Phosphatase Detection Kit	100 assays	SCR004
EmbyroMax ES Cell Qualified 0.1% Gelatin Solution	500 mL	ES-006-B
DMEM/F12, with HEPES, L-Glutamine	500 mL	DF-041-B
Stericup-GP 0.22 µm PES 150 mL RS Filter Unit	12 pk	SCGPU01RE
Anti-TRA-1-60	100 µg	MAB4360

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