Miltenyi Biotec

StemMACS™ iPS-Brew XF enables flexible feeding schedules

Background

Standard culture protocols for human pluripotent stem cells (hPSCs) require daily media replacements, thereby causing researchers to routinely work on weekends. When using conventional PSC culture media, any deviation from these standard protocols can lead to increased spontaneous cell differentiation, slower growth rates, and down-regulation of pluripotency markers.

In this application note we show that StemMACS™ iPS-Brew XF enables more flexible feeding schedules, including the possibility of skipping one or even two feeding days and maintaining hPSC cultures without working on the weekend. Regardless of the feeding schedule used, hPSCs retained the characteristic colony morphology, high expansion rates, and high expression levels of pluripotency markers over five consecutive passages.

Materials and methods

Cell culture

Cells were cultured in StemMACS iPS-Brew XF according to the data sheet. Briefly, cells (iPSC clone K10) were passaged in clumps using StemMACS Passaging Solution and seeded in 6-well plates coated with Matrigel® Matrix. Note that the appropriate splitting ratio must be determined empirically for each cell line. The feeding schedule was modified as indicated in figure 1.

Flow cytometry

Cells were labeled with Anti-TRA-1-60-PE, Anti-SSEA-4-FITC, Anti-SSEA-5-VioBlue®, and Anti-SSEA-1-APC and analyzed by flow cytometry on the MACSQuant® Analyzer 10.

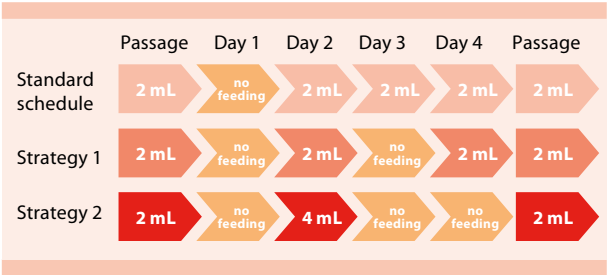

Figure 1: Overview of the different feeding schedules used in this application note. All strategies are based on a 5-day splitting interval. Indicated are the feeding days and media volumes used.

Results

StemMACS™ iPS-Brew XF maintains typical morphology of hPSC colonies regardless of feeding frequency

Colony morphology is an important indicator for the quality of hPSC cultures. Regardless of the feeding schedule used, colony fragments attached in a normal way after passaging with StemMACS™ Passaging Solution XF in 6-well plates coated with Matrigel Matrix. On all days of the passage, colony growth and size were comparable between the standard feeding schedule and the two strategies with reduced feeding frequency. Figure 2A shows representative images taken on days 1, 3, and 5, by way of example. Colony edges were clearly defined with no signs of spontaneous differentiation and colonies were compact in shape. The colony size was comparable between all three conditions, indicating that the supply with nutrients and growth factors was adequate even without daily media replacements.

Changes in the culture conditions can impact hPSCs either directly or with a time lag of typically three to four passages. To exclude long-term effects of the reduced feeding frequency, we monitored the cultures over several consecutive passages. Colony size and appearance were fully comparable between the standard feeding schedule and the two alternative strategies after all passages. Under all three conditions, colonies had defined edges without any sign of differentiation and displayed the typical, compact shape of undifferentiated hPSC colonies. Figure 2B shows results from passages 1, 3, and 5 as examples.

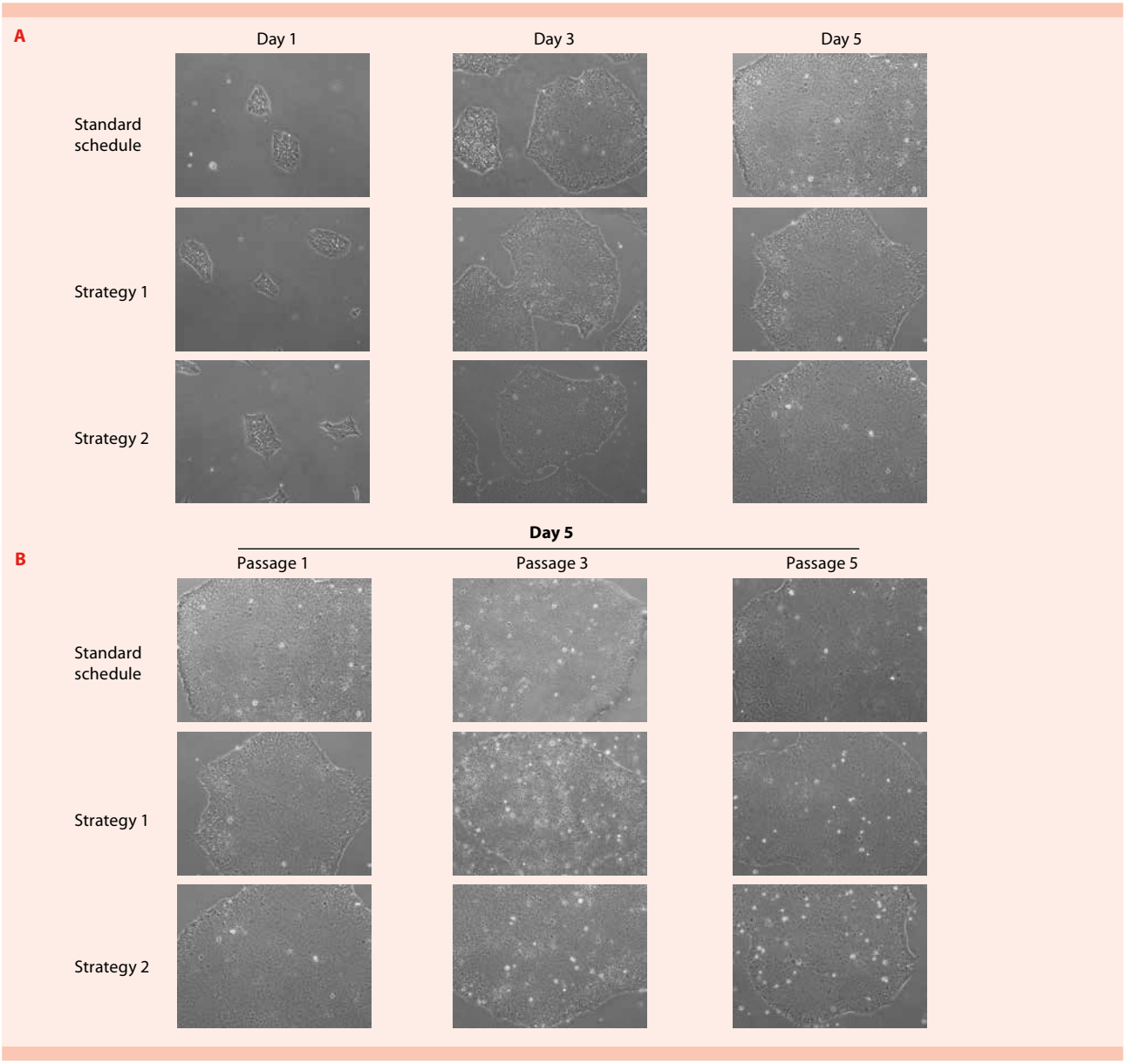

Figure 2: Colony morphology of hPSCs cultured by using different feeding schedules. Cells were cultured as indicated in materials and methods. (A) Light microscopic images were taken after 1, 3, and 5 days of culture. (B) Images were taken on day 5 after 1, 3, or 5 passages.

StemMACS™ iPS-Brew XF supports normal, high hPSC expansion rates even with reduced feeding frequency

Constantly high expansion rates are an important indicator for hPSC culture quality. To ensure that the reduced feeding frequency does not affect proliferation rates, cell counts were determined after each passage. The resulting doubling times were compared between the standard feeding schedule and the two strategies with reduced feeding. Skipping every other feeding day (strategy 1) or even two feeding days (strategy 2) did not alter the expansion rate of the culture over the five passages examined (fig. 3).

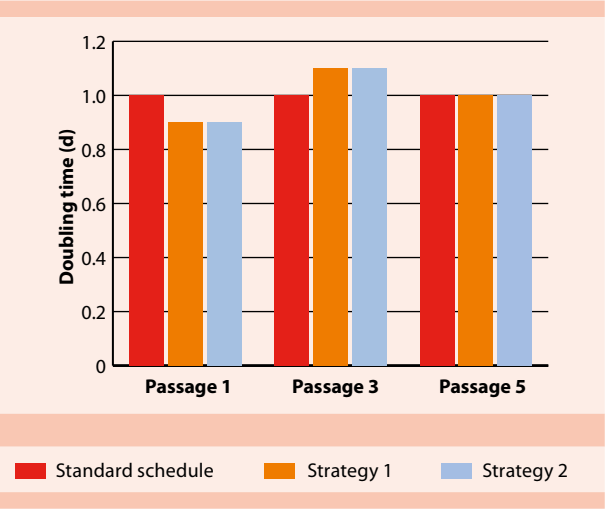

Figure 3: Expansion rates of hPSCs cultured by using different feeding schedules. Cells were cultured as indicated in materials and methods. Cell counts were determined after each passage by flow cytometry using the MACSQuant Analyzer 10, and doubling times were calculated accordingly.

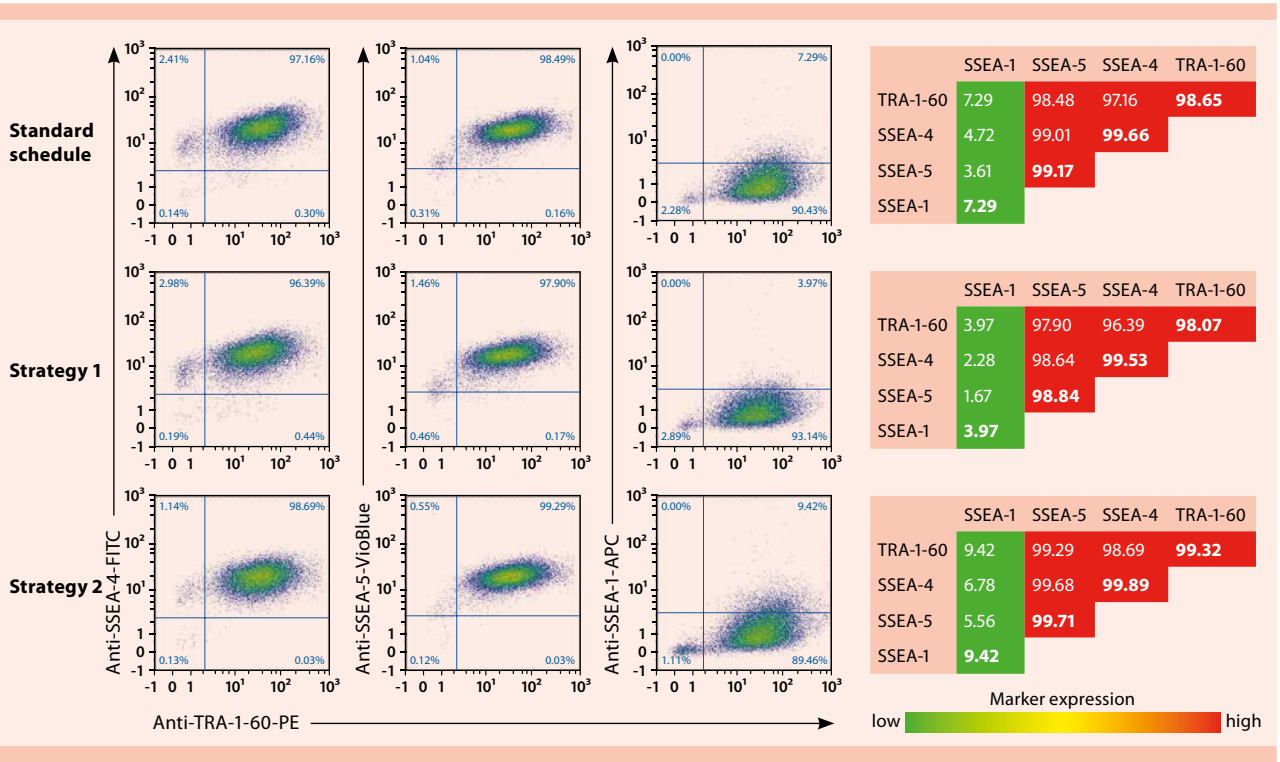

Figure 4: Phenotypes of of hPSCs cultured by using different feeding schedules. Cells were cultured as indicated in materials and methods. After five passages, cells were labeled with Anti-TRA-1-60-PE, Anti-SSEA-4-FITC, Anti-SSEA-5-VioBlue®, and Anti-SSEA-1-APC and analyzed by flow cytometry on the MACSQuant® Analyzer 10. Numbers in the heat map indicate percentages of single-positive (bold numbers) and double-positive cells.

StemMACS™ iPS-Brew XF maintains normal, high expression levels of pluripotency markers in hPSCs cultured with reduced feeding frequency

The phenotype of hPSCs cultured under the three feeding conditions was analyzed by flow cytometry after passages 1, 3, and 5. Figure 4 shows the results from passage 5 as an example. Cells expressed the pluripotency markers, TRA-1-60, SSEA-4, and SSEA-5, at high levels, while the expression level of the differentiation marker SSEA-1 remained low. To facilitate comparison of the different feeding conditions, heat maps were generated to visualize the expression profiles: Red fields indicate high expression levels and green fields low expression levels. Regardless of the feeding schedule used, cultures retained the same expression profile, characteristic of undifferentiated pluripotent stem cells.

Conclusion

StemMACS iPS-Brew XF allows users to skip one or even two feeding days without affecting hPSC culture quality. The schedules with reduced feeding fully maintain the major characteristics of hPSC cultures:

- typical colony morphology,
- high proliferation rates,
- high expression levels of the pluripotency markers, TRA-1-60, SSEA-4, and SSEA-5,
- low levels of the differentiation marker SSEA-1.

MACS Product	Order no.
StemMACS iPS-Brew XF	130-104-368
StemMACS Passaging Solution XF	130-104-688
StemMACS Y27632	130-103-922
StemMACS Thiazovivin	130-104-461
Anti-TRA-1-60-PE, human	130-100-347
Anti-SSEA-4-FITC, human	130-098-371
Anti-SSEA-5-VioBlue, human	130-106-657
Anti-SSEA-1-APC, human	130-104-937
MACSQuant Analyzer 10	130-096-343

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