FULL-TIME PHYSIOLOGIC TEMPERATURE FOR CELL HANDLING REDUCES TRYPSINIZATION TIME OF HUMAN MSC

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Abstract

Traditional cell handling imposes many abrupt temperature changes for human mesenchymal stem/stromal cells (MSC) for routine cell passaging. Even if solutions are warmed before use, the temperature of the room air biological safety cabinet is non-physiologic. The floor of the BSC and the pipettes used to transfer fluids are not at 37ºC, the temperature at which trypsin has maximum enzymatic activity. Traditional trypsinization protocols call for washing the adherent cell layer and adding trypsin at room temperature, then moving the cell cultures into the incubator to slowly warm over the trypsin incubation period. This introduces a tremendous amount of variability into the exposure of cells to peak trypsin activity. In the Xvivo barrier isolator, conditions are at a constant 37ºC. The atmosphere, the processing chamber floor, the pipettes, and the solutions are all at body temperature. When the trypsin is introduced to the cells, it is immediately and maximally active. We set out to determine the optimal time for trypsinization of MSC when all materials are at optimum temperature. We seeded human bone marrow MSC into T-75 flasks. At approximately 70% confluence, we performed routine cell passaging, either at room temp in a traditional process, or in a barrier isolator where the air, the floor, the pipettes, and all solutions were at a constant 37ºC. Cell detachment was monitored microscopically and trypsin was inactivated immediately after detachment. In room temperature conditions, there was greater variability in times for complete cell detachment. In the Xvivo, at a constant 37ºC, MSC fully detached in less than three minutes. We also found greater reproducibility of trypsinization times under constant physiologic temperature conditions. Viability, as determined by trypan blue exclusion, was not changed at reduced trypsinization times as compared to room air handled cells. We conclude that constant temperature conditions yield reduced trypsinization times, increased cell viability, and reduced variability in viable cell yield between batches of MSC.

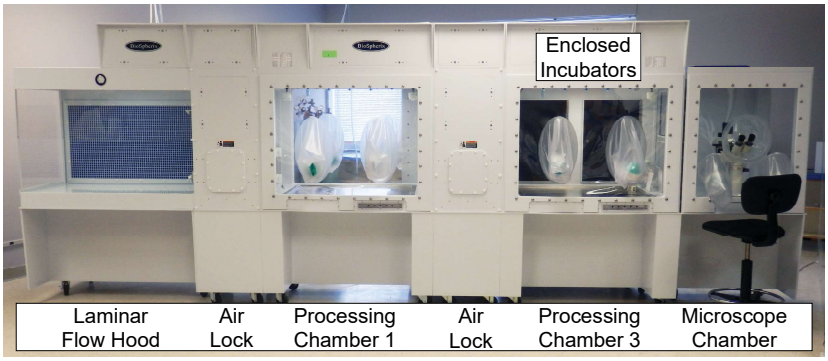
Background

- Trypsinization processes often require moving cells between different temperatures, adding variability
- Human MSC in standard culture and handling conditions behave very differently from MSC in vivo[1]
- We previously showed that part-time room air conditions hindered MSC growth[2,3]
- The Cytocentric barrier isolator warms the air, the processing chamber floor and all materials to 37ºC, maintaining constant conditions for cells even during cell handling.

Objectives

- Trypsinize human MSC in three different conditions: constant room temp, room temp handling with 37ºC incubation, and constant 37ºC
- Determine if there were differences in trypsinization time, cell viability and cell yield

Experimental Design

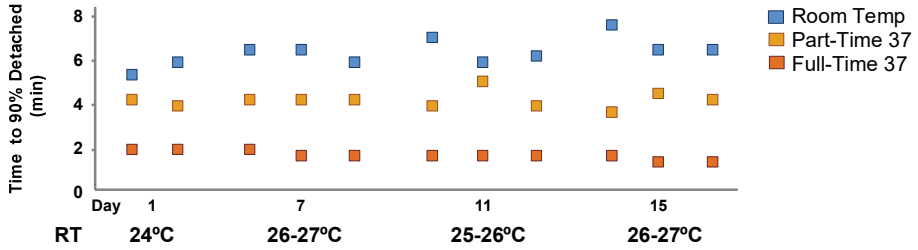


Temperatures	Room Temp	Part-Time 37	Full-Time 37
Handling	RT (Variable)	RT (Variable)	37
Incubation	RT (Variable)	37	37

Figure 1. Experimental Design. Culture of human MSC cultures was performed in a Cytocentric XVIVO barrier isolator. The internal atmosphere was provided by medical-grade, tanked gases, continuously HEPA-filtered and separated from room air. Air and floor of Processing Chamber 3 (PC3) was a constant 37ºC. PC1 was equilibrated to RT. Human MSC (Lonza, GmB) were cultured in 9 T-75 flasks in Lonza Poietics MSC medium and additives and passaged twice a week. Cultures were rinsed with PBS (Sigma) and incubated with 1ml Lonza trypsin. One set of three cultures was trypsinized in constant RT in PC1. All solutions were equilibrated to the cell handling temp before use. To mimic open room processing, one set of 3 MSC cultures were handled at RT in PC1 and incubated in 37ºC incubators in PC3 for detachment. A third set of cultures were handled and incubated at 37ºC in PC3. Detachment was monitored microscopically. Trypsin was inactivated immediately with complete medium. Trypan blue (Sigma) was used for cell viability tests.

Results

2A. Reduced Time to Trypsinize



B. Reduced Variability of Trypsinization Times

	Mean	St Dev	CV
Room Temp	**6.386**	**0.540**	**0.084**
Part-Time 37	**4.500**	**0.387**	**0.086**
Full-Time 37	**2.272**	**0.175**	**0.077**

T test P-value (Two-tailed)	Full-Time vs Part-Time 37	**4.32E-14**
	Full-Time 37 vs Room Temp	**1.63E-11**

C. Viability of Cells Handled at Constant 37ºC

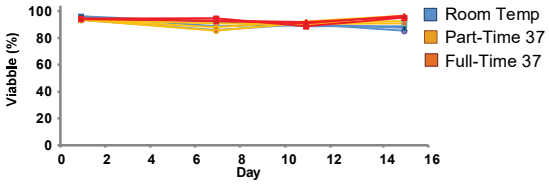


Figure 2. (A) Time to 90% cell detachment during trypsinization was reduced by handling cells at full-time 37ºC (B) Variability in trypsinization times was reduced at full-time 37ºC compared with part-time 37ºC or full-time room temperatures (Two-tailed T test (Excel, Microsoft). ANOVA analysis also showed significant differences between groups. (C) Viability of Cells Handled at Full-Time 37ºC was not decreased compared with Part-Time 37ºC or Room Temp. This study was performed twice. Results of one are shown.

Conclusion

Human MSC handled at constant 37ºC during trypsinization detached more quickly and more reproducibly than MSC trypsinized in variable room air conditions.

References

1.Marfy-Smith, S.J. and C.E. Clarkin, Are Mesenchymal Stem Cells So Bloody Great After All? STEM CELLS Translational Medicine, 2017. 6(1): p. 3-6.
2. Henn, A., Darou, S. & Yerden, R. Physiologically Relevant Oxygen during cell Handling as Well as Incubation Enhances the Growth of Human Mesenchymal Stromal Cell Cultures. International Society for Stem Cell Research (2016).
3. Henn, A., Farrell, G., Darou, S. & Yerden, R. Shock from Exposure to Room Air Conditions Alters Individual Mesenchymal Stem Cell Fate, Population Dynamics, and Batch Yields. International Society of Cell Therapy North America (2017).