Cell culture

Using TrypLE Express Enzyme for dissociation of adherent primary cells

Introduction

Primary cells tend to be more difficult to culture than established cell lines. This can be due to factors such as the onset of senescence as well as the time needed for primary cells to adapt to their new cell culture environment. Therefore, dissociation methods that do not negatively affect the growth of cells in culture must be utilized when passaging primary cells.

Comparing dissociation using TrypLE Express Enzyme to the currently recommended method

Currently, it is recommended that primary cells be dissociated with Gibco™ Trypsin/EDTA Solution and then diluted with Gibco™ Trypsin Neutralizer Solution. To verify the effectiveness of Gibco™ TrypLE™ Express Enzyme as a gentle alternative for dissociating primary cells without compromising cell health or growth, three types of primary cells were cultured for three passages and dissociated with either the trypsin/EDTA solution or TrypLE Express Enzyme. Following dissociation, the trypsin/EDTA solution was diluted with Trypsin Neutralizer Solution, and TrypLE Express Enzyme was diluted with complete medium. At each passage, initial confluency, dissociation time, cell count, viability, confluency of the replated cells, and morphology of the replated cells were observed with two replicate flasks in each group. For all three cell types tested, no difference was observed between the dissociation methods across all three passages for any metric measured. The data of one representative passage are shown for human aortic endothelial cells (Figure 1), neonatal human dermal fibroblasts (Figure 2), and human coronary artery smooth muscle cells (Figure 3).

The results indicate that TrypLE Express Enzyme is just as effective as the trypsin/EDTA solution for dissociation of adherent primary cells in culture. Advantages of TrypLE Express reagents include:

- Gentle on cells—dissociate strongly adherent cells while retaining cell surface markers
- Animal origin–free (AOF)—TrypLE Express Enzyme is free of animal-derived components, a requirement for some applications
- No inhibitor required to inactivate—inactivation is achieved by dilution alone
- Room temperature storage—simplified workflow and sustainability
- Save time and resources—no thawing or aliquoting saves ~45 minutes in experiment prep time and ~200 g of plastic (20 aliquots)
- Sustainability—transported at ambient temperature in cardboard instead of expanded polystyrene (EPS) foam

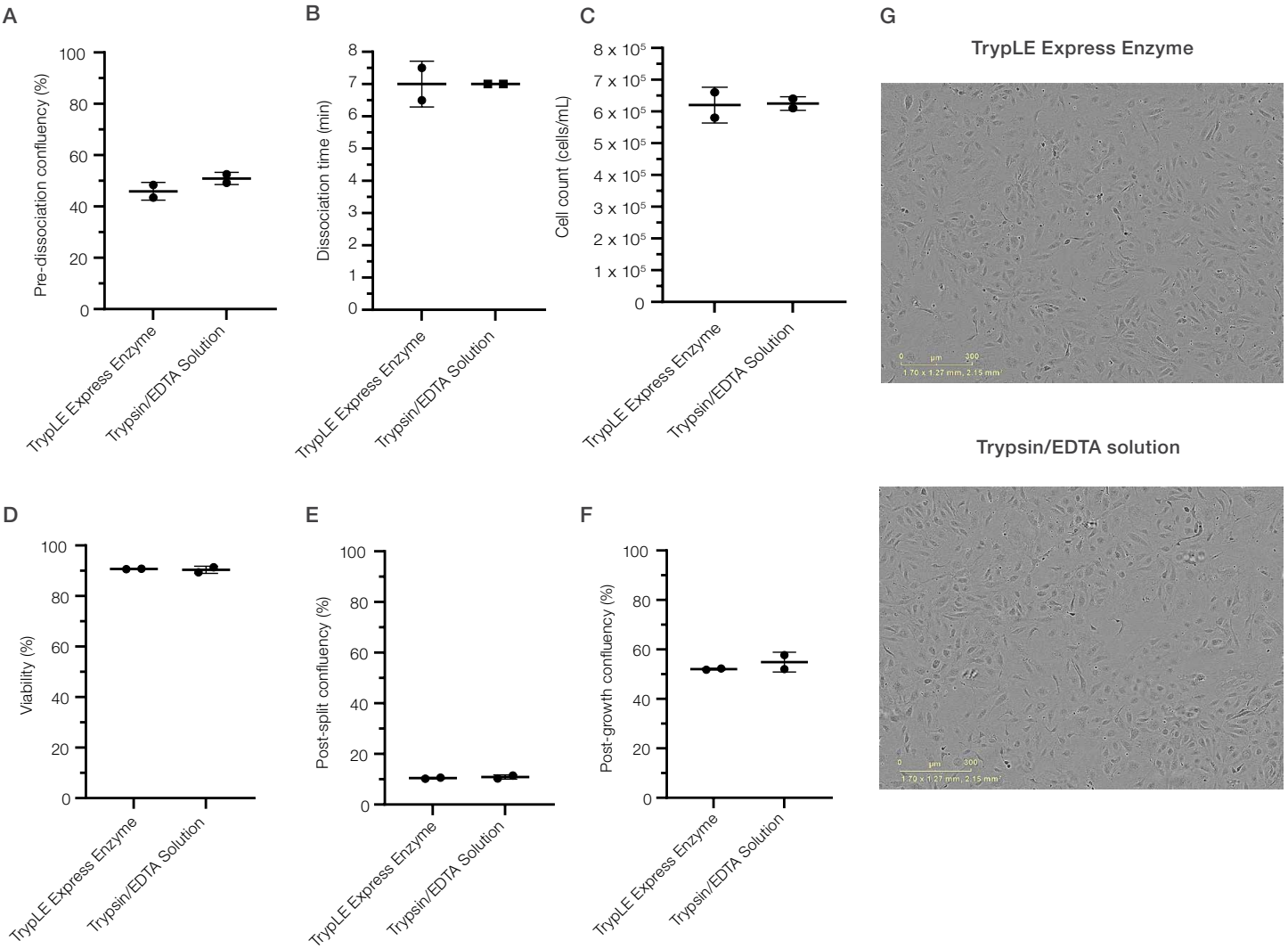

Figure 1. Representative data (N = 2 flasks) from one of three passages of culturing human aortic endothelial cells and dissociating with TrypLE Express Enzyme or trypsin/EDTA solution. Shown are **(A)** pre-dissociation confluency, **(B)** dissociation time, **(C)** cell count, **(D)** viability, **(E)** confluency after dissociation, **(F)** confluency after 4 days of growth when replated, and **(G)** morphology after 4 days of growth when replated.

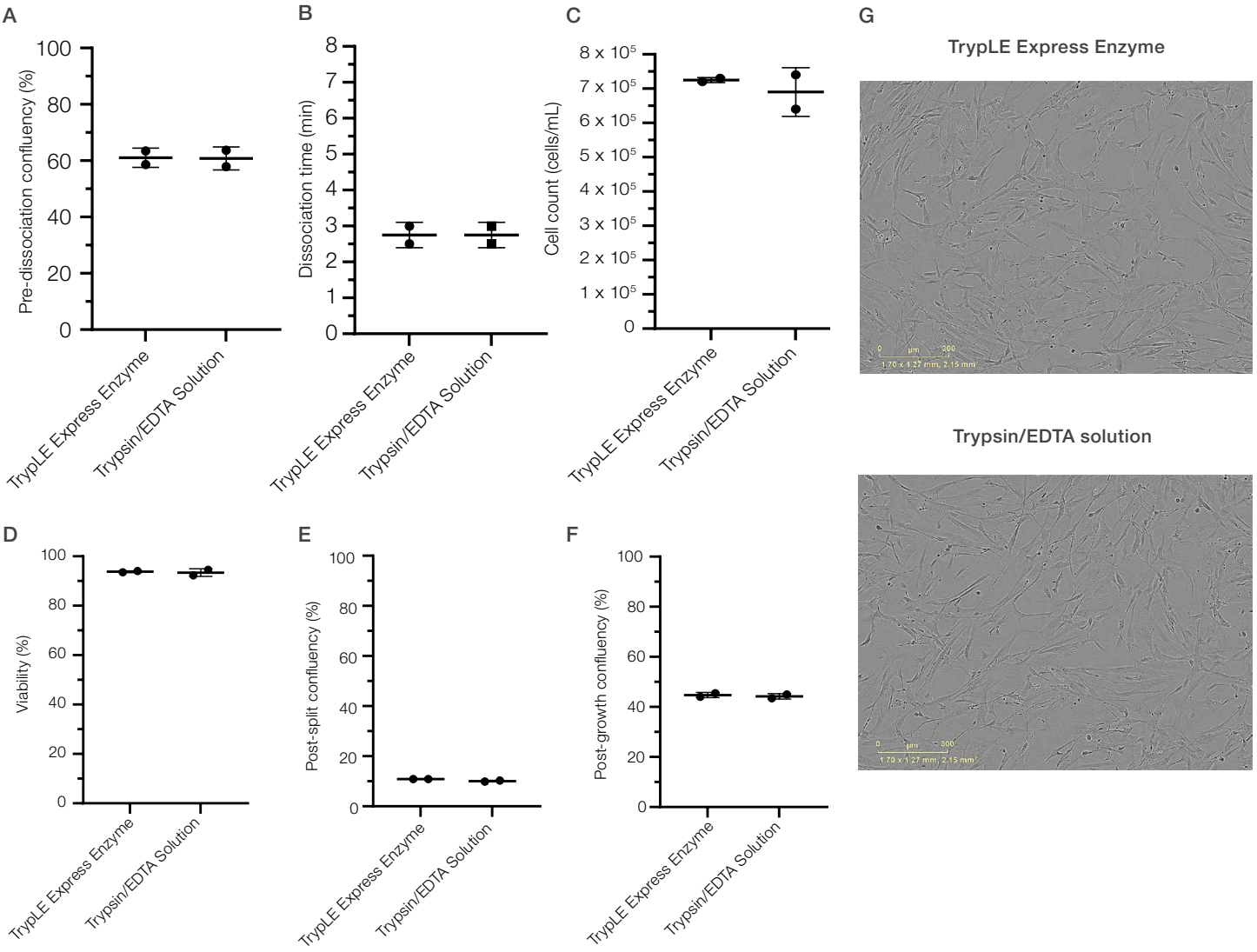

Figure 2. Representative data (N = 2 flasks) from one of three passages of culturing neonatal human dermal fibroblasts and dissociating with TrypLE Express Enzyme or trypsin/EDTA solution. Shown are **(A)** pre-dissociation confluency, **(B)** dissociation time, **(C)** cell count, **(D)** viability, **(E)** confluency after dissociation, **(F)** confluency after 4 days of growth when replated, and **(G)** morphology after 4 days of growth when replated.

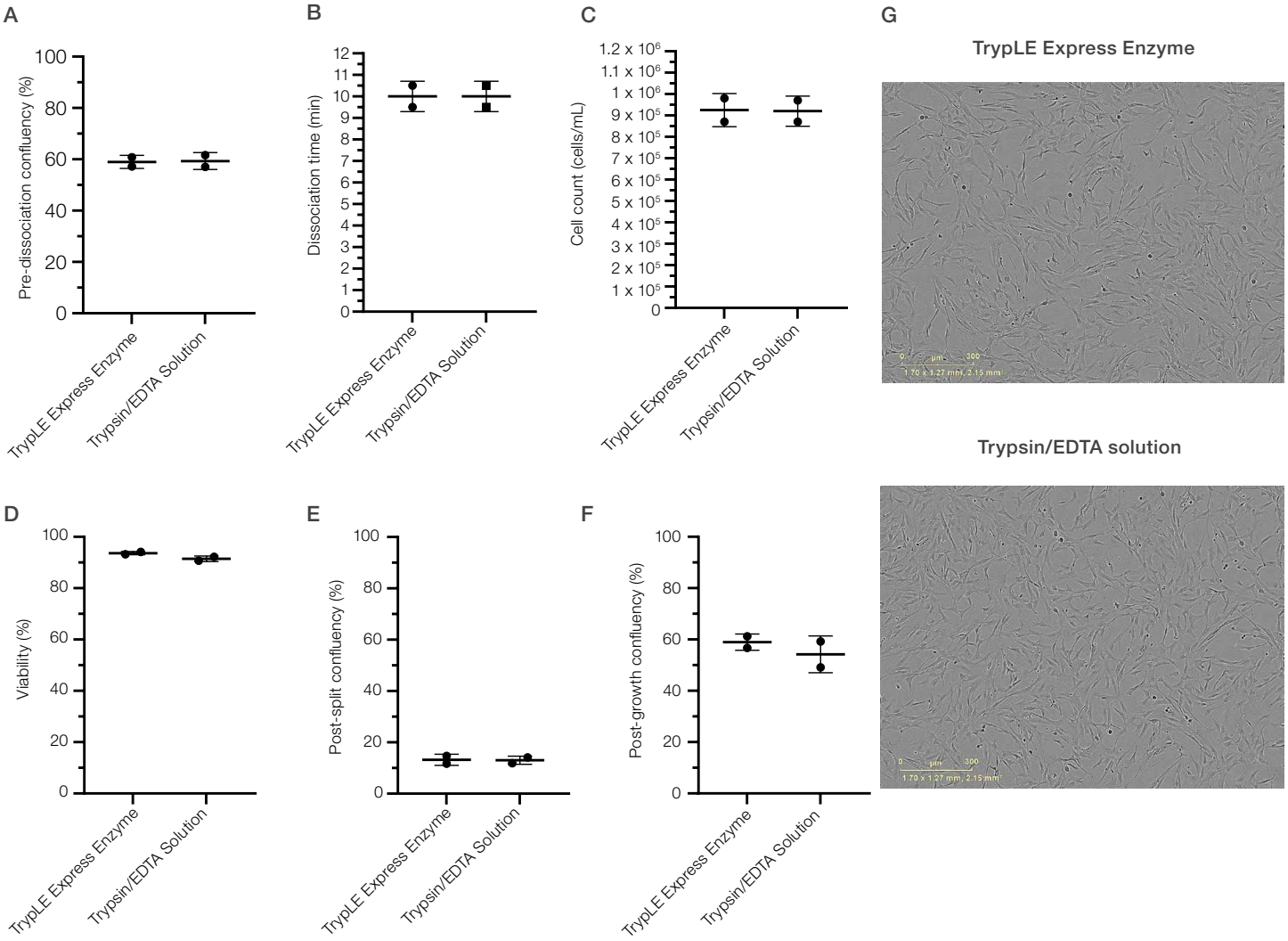

Figure 3. Representative data (N = 2 flasks) from one of three passages of culturing human coronary artery smooth muscle cells and dissociating with TrypLE Express Enzyme or trypsin/EDTA solution. Shown are **(A)** pre-dissociation confluency, **(B)** dissociation time, **(C)** cell count, **(D)** viability, **(E)** confluency after dissociation, **(F)** confluency after 3 days of growth when replated, and **(G)** morphology after 3 days of growth when replated.

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