

Optimization of Harvesting and Dissociation Strategies for Alveolar Organoids

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INTRODUCTION

Alveolar organoids are advanced 3D models that more accurately mimic multicellular complexity and structural arrangement of native alveolar tissue in comparison to other conventional in vitro systems. Although protocols and reagents for harvesting exist, optimized methods for harvesting cell-line-derived alveolar organoids remain underexplored.

AIMS

1. Develop and annotate a reproducible pilot protocol for organoid harvesting and dissociation. Using this protocol, culture organoids, harvest them, embed in collagen-I, re-harvest, and evaluate cell viability.
2. From single-cell suspensions, assess membrane protein integrity and cell-type-specific marker expression using immunofluorescence staining.

MATERIALS & METHODS

The finalized pilot protocol for organoid harvesting and dissociation was as followed;

- Matrigel was mixed on ice with DMEM in a 2:3 ratio and left in a 37°C incubator for an hour to solidify
- A suspension of A549 and MRC5 cells in a 10:1 ratio in 5% Matrigel/culture medium was triturated and embedded on top of solidified Matrigel
- Organoids were left to differentiate for 11 days, changing 5% Matrigel/culture medium every 48 hours, and brightfield images taken at 48 hours, day 4, day 7, and day 11
- On day 11, organoids were removed from the Matrigel by digestion with Organoid Harvesting Solution for either a 15- or 30-minutes incubation period
- For control 1, culture medium was removed and 250 µl of 10% Alamar Blue solution was adding directly on top of the Matrigel and triturated to mechanically digest the Matrigel. For Control 3, culture medium was removed, 200 µl of cold PBS was added, triturated, centrifuged for 5 minutes at 200 x g, removed supernatant, and resuspended in 250 µl of 10% Alamar Blue solution
- Collected organoids were either resuspended in PBS for cell count, Alamar blue for a viability assay, or in rat tail collagen type 1 gel
- Additional organoids were collected and tagged with antibodies for immunofluorescence staining
- Culture media was added on top of organoid embedded collagen 1 gels and left overnight in the 37°C incubator
- Collagen 1 gels were digested by removing culture medium and adding collagenase type 1 for an incubation period of 30 minutes in the 37°C incubator
- Collected organoids were either suspended in PBS for cell count, Alamar blue for a viability assay, or incubated with Trypsin-EDTA, TrypLE, or Accutase to obtain a single cell suspension
- For the collagen control group, culture medium was removed, 10% Alamar Blue solution was added directly on top of the organoid embedded collagen gels and triturated
- Cell countess was used for cell counts once a single cell suspension was obtained and suspended in Alamar blue for a viability assay

Brightfield Images of Alveolar Organoid Differentiation

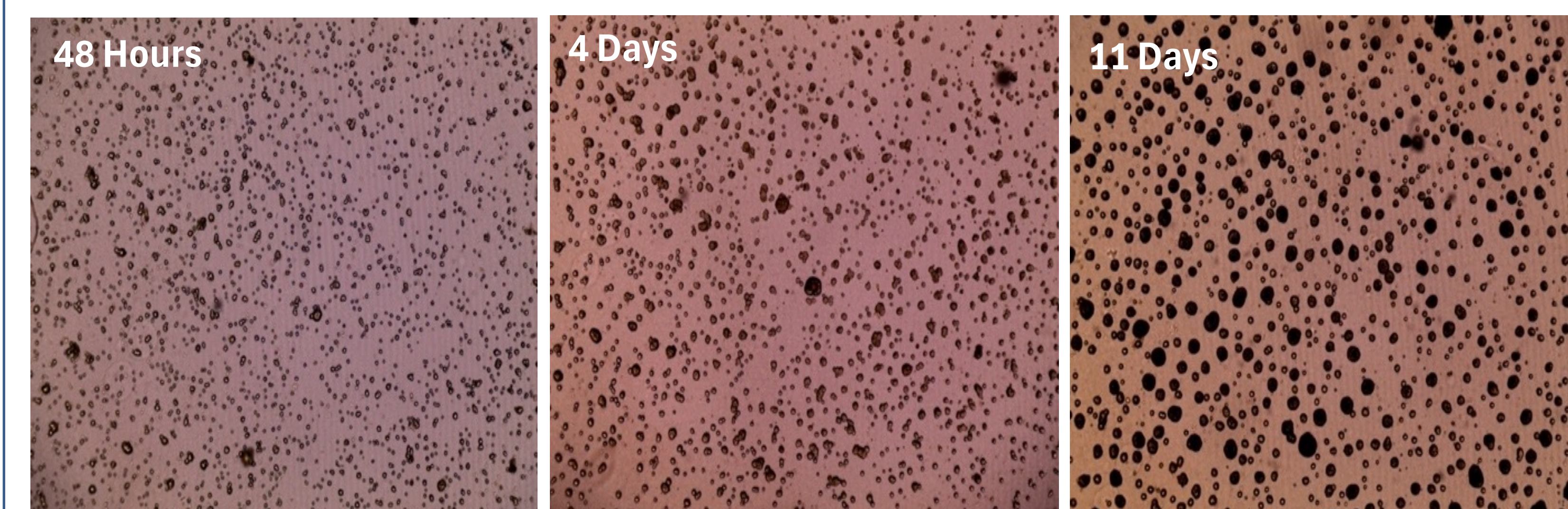


Figure 1. Brightfield images tracking alveolar organoid derived from A549 and MRC5 cells differentiation over the span of 11 days.

RESULTS

Viability Assays From Matrigel Digestion

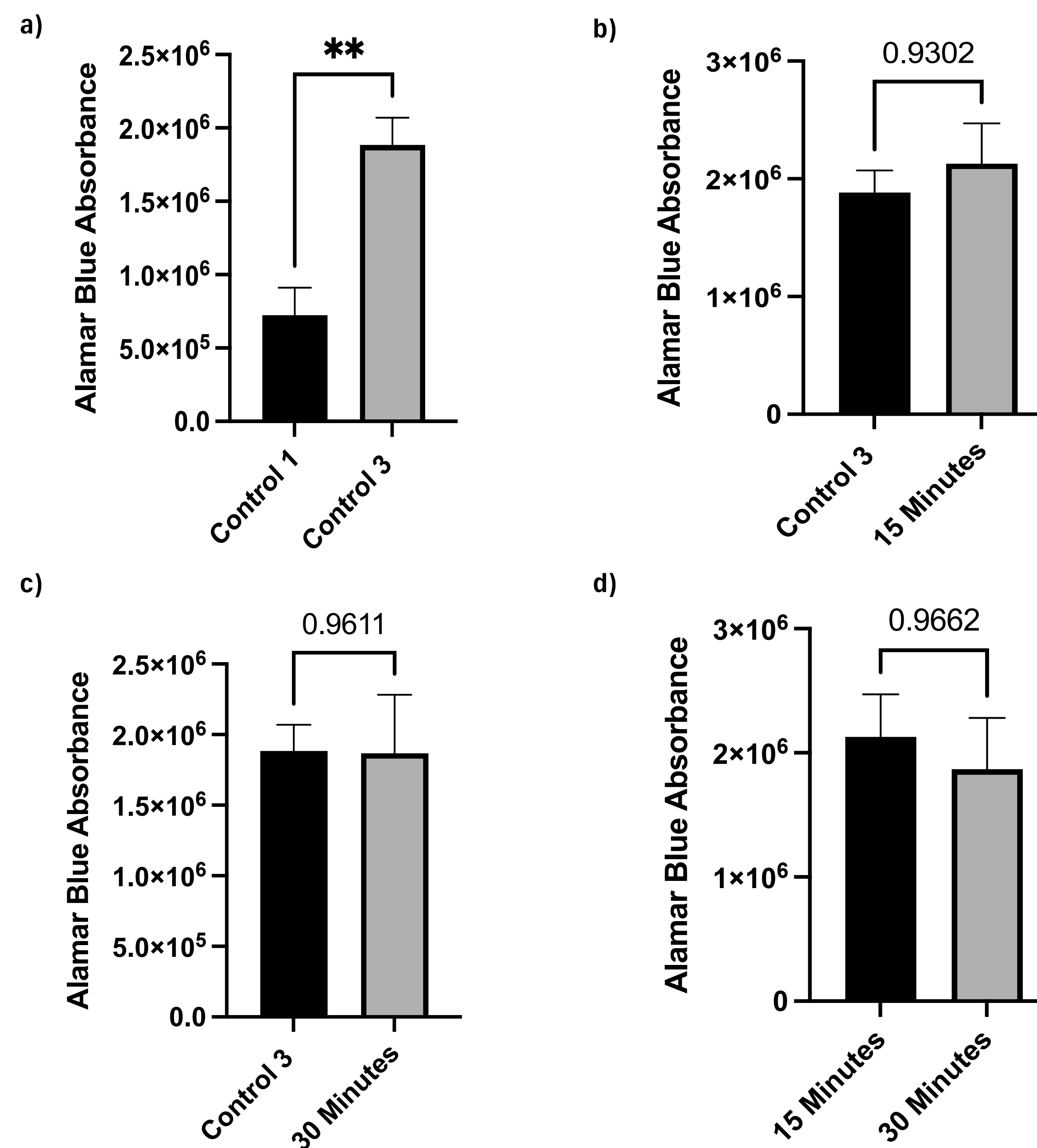


Figure 2. Alveolar organoid cell viability following Matrigel digestion protocol assessed by Alamar Blue viability assay. a) Viability assays showed a significant difference between Control 1 and Control 3 ($p = 0.0076$). Control 3 having shown a more favourable viability. b) Viability assays showed no significant difference between Control 3 and 15-minute group. c) Viability assays showed no significant difference between Control 3 and a 30-minute group. d) Viability assays showed no significant difference between the 15-minute group and the 30-minute group.

Viability Assays From Collagen Digestion and Single Cell Suspension

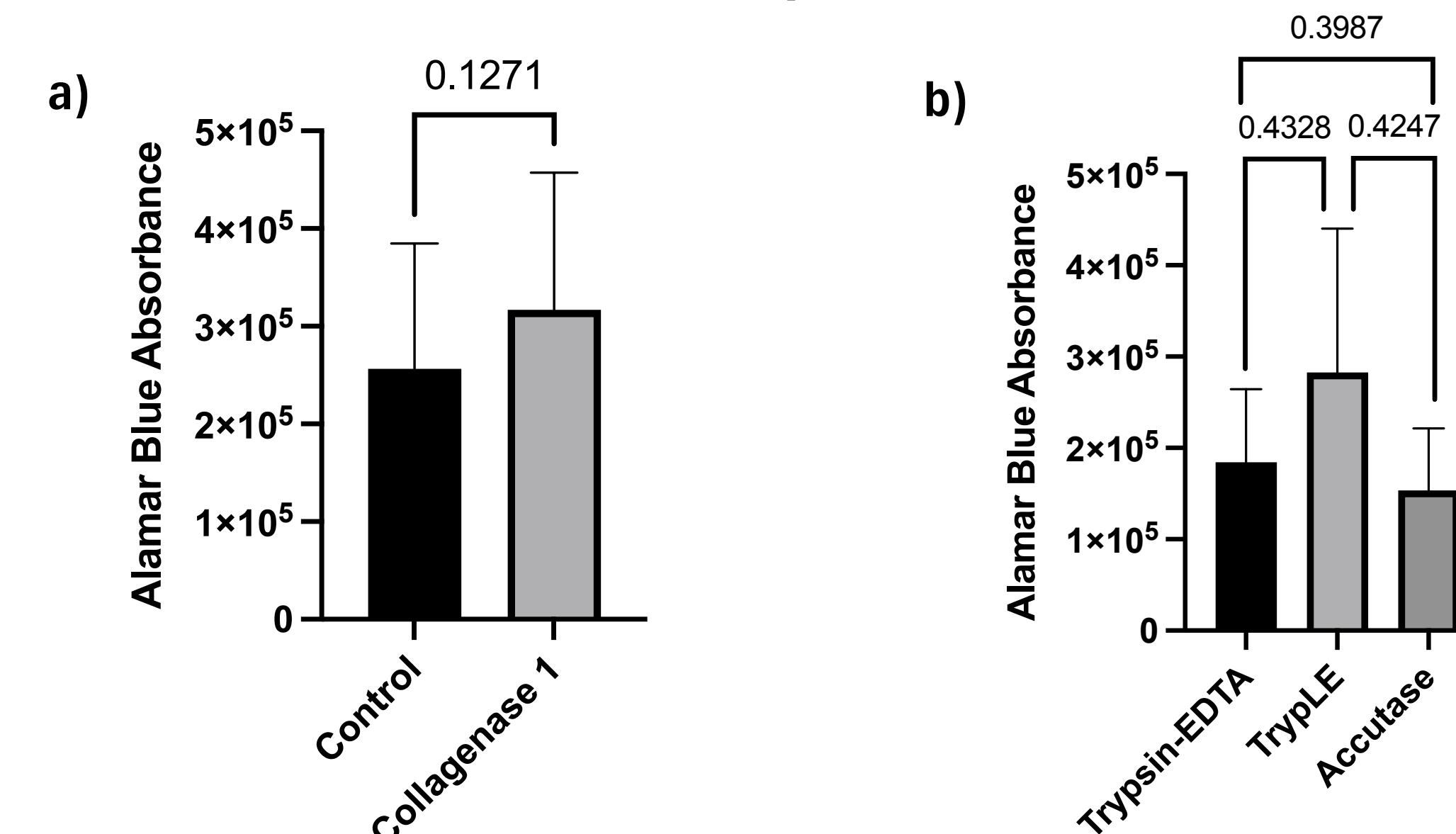


Figure 3. Alveolar organoid cell viability following collagen gel digestion protocol and obtaining a single cell suspension from organoids assessed by Alamar Blue viability assay. a) Viability assays shows no significant difference between Control and Collagenase 1. b) Viability assays showed no significant difference between Trypsin-EDTA, TrypLE, and Accutase.

RESULTS

Characterization of Alveolar Organoids by Immunofluorescences (IF)

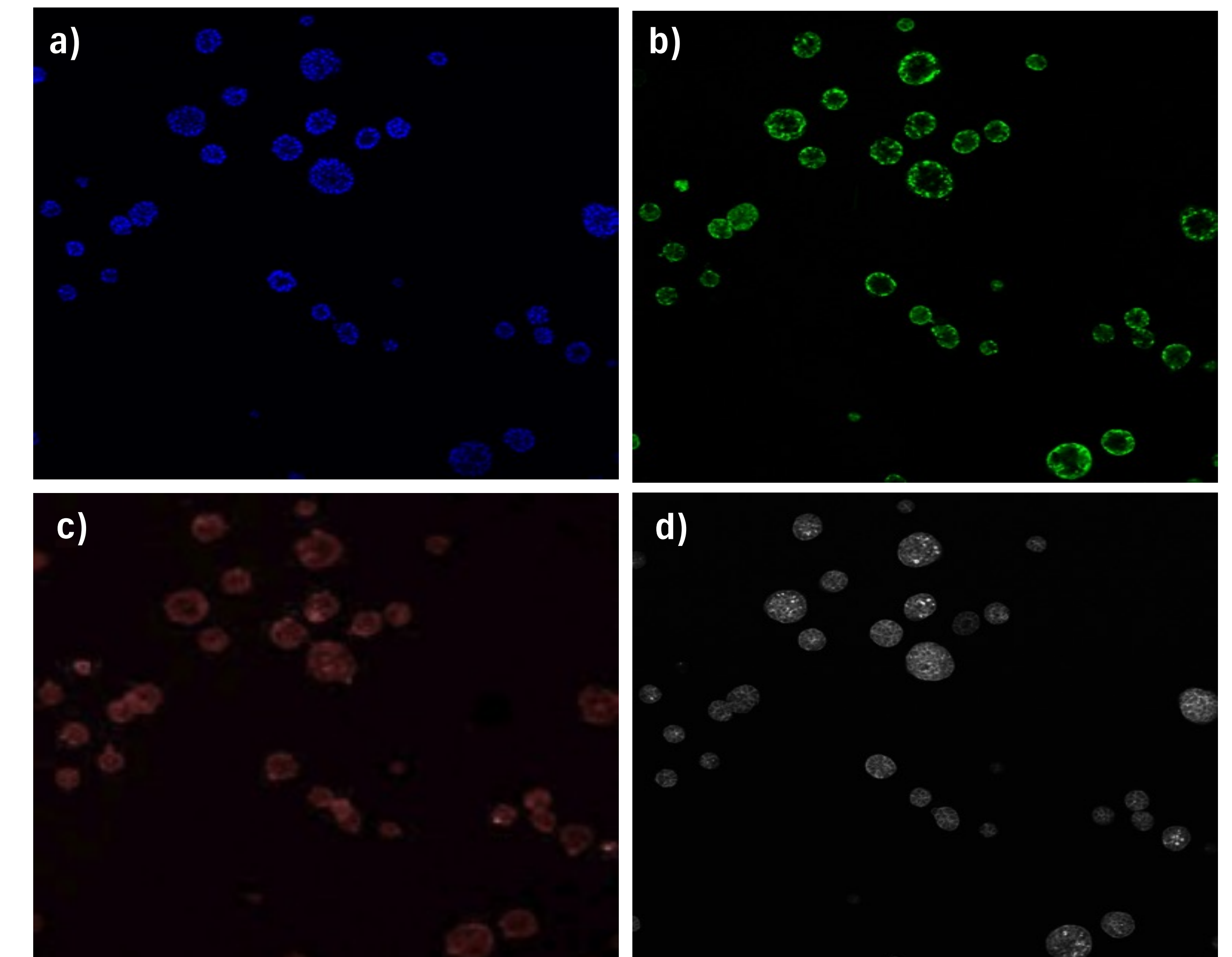


Figure 4. Cell-type-specific markers individually expressed in A549-MRC5 derived organoids. a) DAPI (blue), shows positive expression of cellular nuclei. b) AQP-5 (green), shows positive expression of A549 epithelial cells. c) T-E7 (red), shows positive expression of MRC5 fibroblast cells. d) Phalloidin (white), shows positive expression of F-Actin found in the cytoskeleton of both A549 and MRC5 cells.

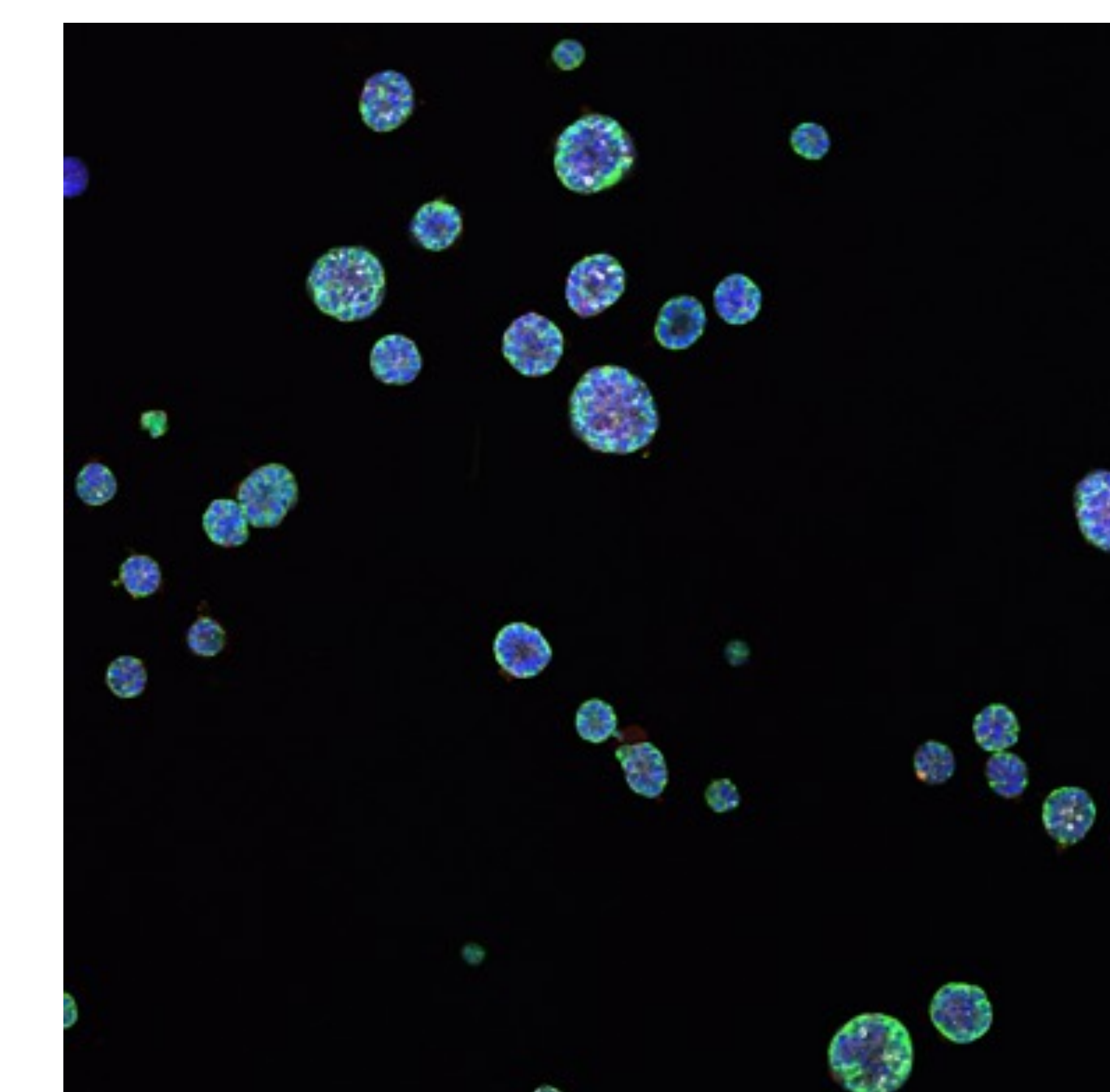


Figure 5. Alveolar organoids derived from A549 and MRC5 cells expression were accessed with cell-type-specific marker using immunofluorescence staining. Cells were stained with four different cell markers; DAPI (blue), AQP-5 (green), T-E7 (red), and Phalloidin (white).

CONCLUSIONS

- No significant differences in viability were observed across varying durations of Matrigel digestion
- Collagen-based matrices were readily digested with minimal optimization using collagenase I
- No clear advantage was observed among Trypsin-EDTA, TrypLE, and Accutase in generating single-cell suspensions, indicating that optimal conditions regarding the use of these reagents require further investigation
- These optimized protocols provide a robust and reproducible framework that will enable reliable downstream analyses in diseased lung models, supporting more precise mechanistic interrogation and translational application in future studies.

