Optimized Protocols for Enzymatic Isolation of Viable Cells from Lung-Fibroblast-Embedded 3D Hydrogels

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INTRODUCTION

Hydrogels are cross-linkable polymer networks that form 3D structures to mimic the extracellular matrix (ECM) of different tissues, such as the lungs.[1] A commonly used hydrogel is Gelatin Methacrylate (GelMA). Lung fibroblasts are the main cells that produce and remodel the lung-ECM. Lung fibroblast embedded hydrogel models create 3D cell cultures used to study the ECM stiffness, functional repair phenotype of fibroblasts and closely mimic the *in vivo* environment. However, there are no optimized methods for isolating viable cells from lung-fibroblast-embedded hydrogels.

AIMS

This study aimed to research, identify and optimize 2 enzymatic protocols for the recovery of whole viable lung fibroblasts from GELMA hydrogels to enable downstream molecular assays.

METHODS

Through an extensive literature review we chose 2 enzymatic protocols to optimize which included, the use of Collagenase 1 (Cell-Collect-G) & Trypsin/EDTA enzyme isolation.

- 500 µL GelMA hydrogels with 500K MRC-5 lung fibroblasts were UV-cured to establish 3D hydrogel models.
- Hydrogels were digested with protocols adapted from either the manufacturer for Collagenase (Cell-Collect-G (Cellink®)) or through our research for Trypsin/EDTA.
- Trypan blue exclusion and the Countess cell counting system was used to determine total cell counts and % viability of cells isolated.
- The Pierce Rapid Gold BCA Protein Assay kit was used to determine the protein concentrations from isolated cells.

Methods - GelMA hydrogel constructs

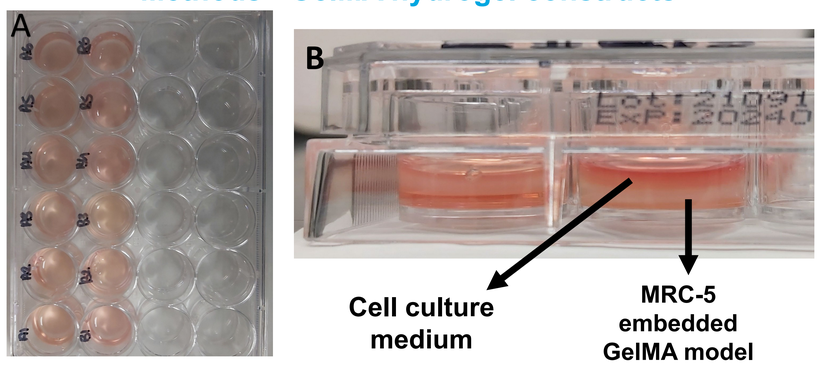

Figure 1: Lung fibroblast-embedded GelMA hydrogel constructs & isolation. MRC-5 cells were seeded in GelMA hydrogels UV-cured and incubated with, 500 µL of cell culture medium for 48hrs in standard conditions. A) Top-view of constructs, B) Side-view of constructs.

Methods – Digestion of GelMA constructs

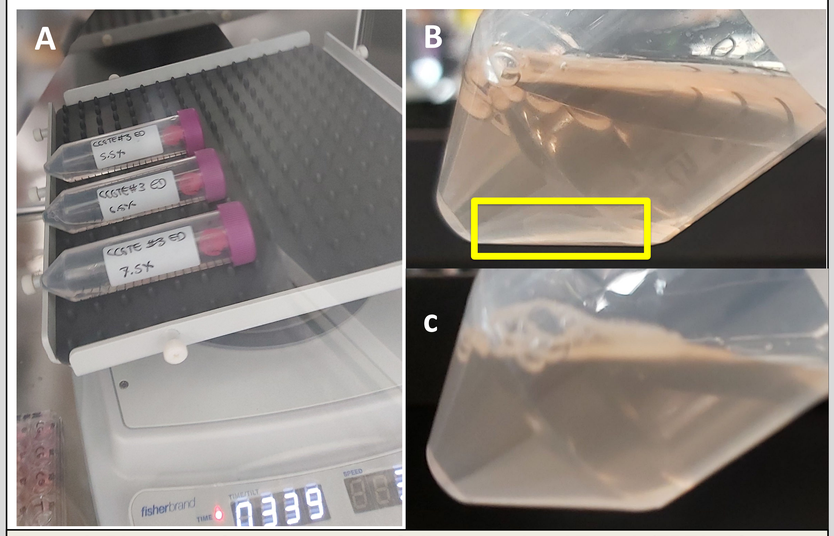

Figure 2: Digestion of constructs with Collagenase I and Trypsin/EDTA. After incubation, MRC-5-GelMA gels were subjected to Collagenase I and Trypsin/EDTA isolation for 1hr per manufacturer's instruction and our research. A) Image of GelMA construct in Collagenase I and Trypsin on Rocker, B) Representative Image of GelMA constructs in Collagenase I at t=0, C) Representative Image of GelMA constructs after Collagenase 1 digestion at t=1hr.

RESULTS

Higher average cell counts achieved with Collagenase I for 5.5 and 6.5% GelMA constructs

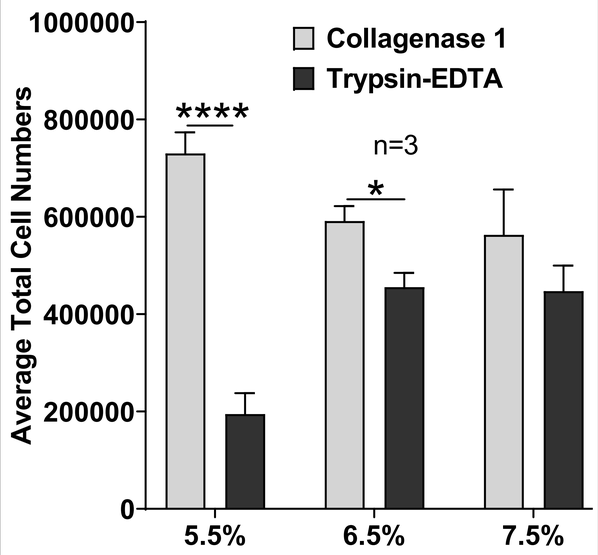

Figure 3: Digestion of constructs with Collagenase I yields higher average total cell numbers for 5.5 and 6.5% GelMA hydrogels. Cell pellets were obtained from digested hydrogel solutions and immediately counted with Countess cell counting system to obtain recovered cell numbers. Average total numbers of isolated lung fibroblasts using Collagenase I or Trypsin/EDTA from 3 different GelMA concentrations was graphed. ****= P<0.0001, * = p<0.05

Results - Percentage cell viability of isolated lung fibroblasts from GelMA Hydrogels

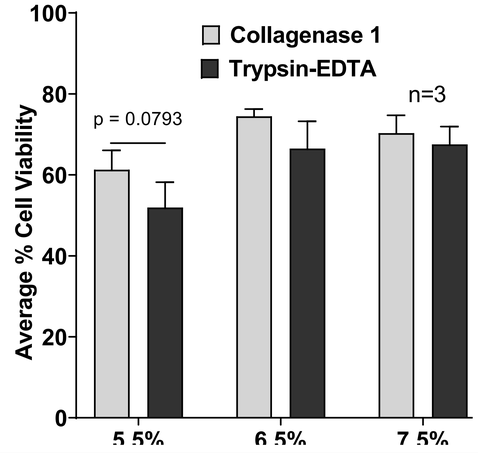

Figure 4: Percentage cell viability of isolated lung fibroblasts from GELMA hydrogels after Collagenase I and Trypsin/EDTA digestion protocols. Average % cell viability of recovered lung fibroblasts were measured using Countess cell counting system from the cell pellets. Trend (p = 0.0793) towards significance detected between Collagenase I & Trypsin/EDTA isolation for 5.5% gels

Results - Similar protein concentrations from lung fibroblasts after Collagenase 1 & Trypsin/EDTA isolations

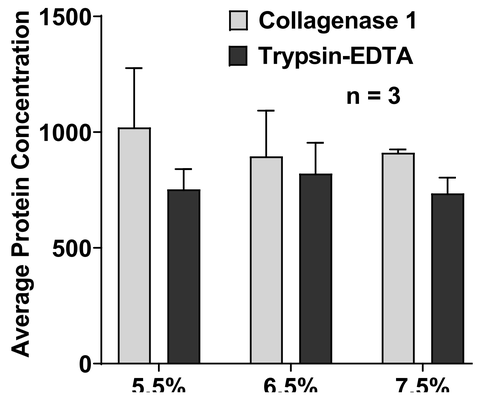

Figure 5: Protein concentrations of isolated lung fibroblasts from GELMA hydrogels after Collagenase I and Trypsin/EDTA digestion protocols. Protein extraction buffer was used to extract proteins from isolated MRC-5 fibroblasts previously embedded in GELMA. Average protein concentration of isolated MRC5 fibroblasts were measured using Pierce Rapid Gold BCA Protein Assay according to Manufacturer's instructions.

CONCLUSIONS

- Collagenase 1 (Cell Collect G) enzymatic isolation protocol aids in isolating significantly higher counts of lung fibroblasts embedded in from GelMA hydrogels of different percentages (5.5 and 6.5%) and may cause higher percentage viability yields in cells compared to the Trypsin/EDTA.
- Both Collagenase 1 & Trypsin/EDTA isolation protocols seem to not have an effect on protein concentration according to our data. However more repeats are needed for conclusions.

REFERENCE

1. Caliari, S. R.; Burdick, J. A. A Practical Guide to Hydrogels for Cell Culture. Nature Methods 2016, 13 (5), 405–414.

PARTNERS: