Optimizing Vascular Tissue Digestion Protocols for scRNA-seq Analysis Preparation

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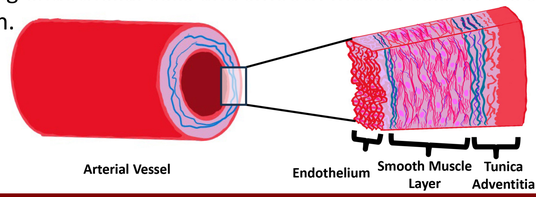
Introduction

Single-cell RNA sequencing (scRNA-seq) has emerged as a vital method for investigating the composition and functionality of cells within both healthy and diseased tissues, allowing us to measure the transcriptomes at the individual cell level. However, the success of scRNA-seq experiments relies heavily on the quality of single-cell suspensions obtained from enzymatic digestion of the tissues. In this study, with a particular focus on the vascular tissues and cell populations, our primary objective is to examine whether varying incubation time during enzymatic digestion could improve the cell yield and viability of our single-cell suspensions. Our specific aim is to refine the enzymatic digestion condition on different segments of the arterial tree and validate the presence of vascular cell types, including endothelial cells and smooth muscle cells in the single-cell solution.



Methods and Materials

Schematic diagram of the experimental procedure

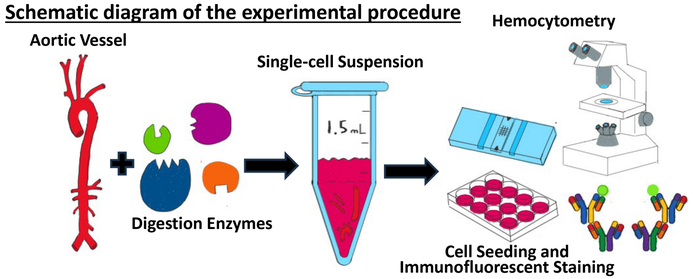
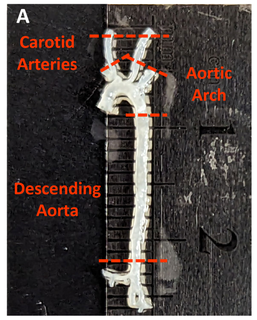


Figure 1: Arterial tissues were isolated from euthanized C57BL6 mice. An enzyme cocktail consisting of Liberase, Hyaluronidase, and DNase I was used to digest the extracellular matrix and remove genomic DNA. Following enzymatic digestion for either 30 or 60 minutes, the digested samples underwent straining to eliminate debris, producing the single-cell suspensions. The number and viability of the isolated cells were determined using a cell-counting hemocytometer. Additionally, the presence of various vascular cell types were assessed through immunofluorescence staining.

Results

Arterial Tissue Cleaning and Enzymatic Digestion

A

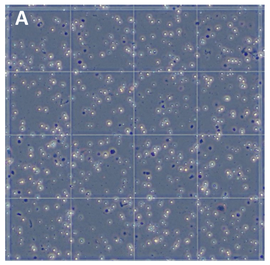


B

	Average Length (mm)
Carotid Arteries	5.66667
Aortic Arch	4
Descending Aorta	12.66667

Figure 2: (A) Arterial tissues were harvested from the carotid bifurcation to the abdominal aorta. Cleaned vessels were sectioned into 3 distinct regions of interest, reflecting their susceptibility or resistance to cardiovascular disease: the carotid arteries, aortic arch, and descending aorta. (B) The dimension of each vessel segment.

A



B $\frac{\text{Cell Counted}}{\text{Squares Observed}} * \text{Dilution factor} * \text{Initial Volume (ml)} * 0.1\ \text{mm}^3$

C $\frac{\text{Live Cells}}{\text{Total Cells}} * 100$

Figure 3: (A) The cells isolated from the arterial tissues were loaded onto a hemocytometer for cell counting. The white circles were viable live cells and the Trypan Blue Dye stained dead cells were dark blue. Irregular dark blue shapes were considered debris. The equation of total cell number (B) and cell viability (C). The bar graphs represent the averages from data collected from three animals.

The Yield and Viability of Isolated Cells in the Single-cell Suspension

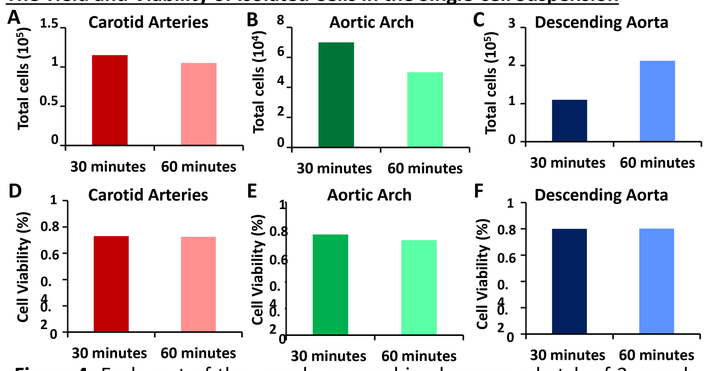


Figure 4: Each part of the vessel was combined among a batch of 3 vessels. Time points to compare were 30- and 60-minute incubation periods with the extracellular matrix digestion enzymes. Fig. ABC depict cell yield, and Fig. DEF depict cell viability.

Immunofluorescence Staining of Vascular cells in the Single-cell Suspension

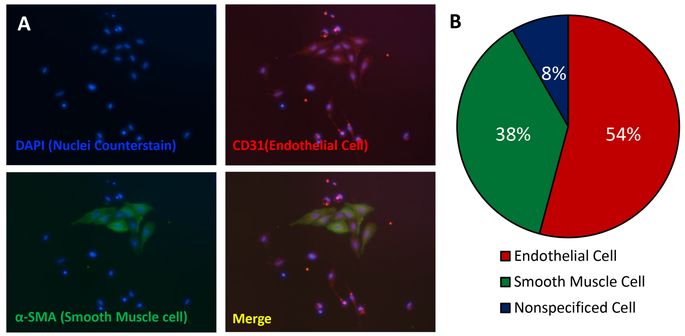


Figure 5: (A) The isolated cells were seeded into a cell culture dish coated with fibronectin to promote cell adhesion. The attached cells were fixed with 4% paraformaldehyde and subsequently subject to immunofluorescence staining of endothelial cells (CD31, green) and smooth muscle cells (alpha-smooth muscle actin, red). DAPI was used to counterstain the nuclei. (B) Distribution of various cell types quantified on fluorescent images

Future Works

We will continue to refine the enzymatic digestion protocol for arterial tissues to enhance yield and cell viability before conducting the scRNA-seq experiments. We will employ flow cytometry with multiple cell markers to validate the composition of vascular cell populations with the tissues. Ultimately, we will apply the optimized protocol to digest vascular tissues obtained from animals affected by atherosclerotic cardiovascular disease and conduct scRNA-seq analysis to the delineate the underlying pathology of atherosclerotic plaque development.

Acknowledgments

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References

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