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December 15, 2019

Dear Dr. Jewhurst,

We would like to submit our new manuscript entitled "**Isolation of Endocardial and Coronary Endothelial Cells from the Ventricular Free Wall of the Rat Heart**" for consideration for publication in JoVE. We believe that the novel method for isolating endocardial cell and coronary endothelial cell presented in our manuscript would be of great interest and value to the diverse readership of JoVE.

Significance
It has been shown that endocardial endothelial cells (EECs) and coronary endothelial cells (CECs) differ in origin, development, markers, and functions. Consequently, these two cell populations play unique roles in cardiac diseases. Current studies involving isolated endothelial cells investigate cell populations consisting of both EECs and CECs, making it infeasible for researchers to determine cell-type specific mechanisms for heart development and diseases. Thus, the ability to isolate EECs and CECs independently from the heart is crucial for cell specific characterization.

Novelty and Strengths
1) We are the first group reporting the protocol for isolating EECs and CECs independently from the rat heart.
2) We used newly reported EECs and CECs specific markers for verification of successful isolation through gene expression analyses.
3) This method maintains cell phenotype characteristics upon isolation, allowing subsequent cell culturing and downstream functional analysis.
4) This method could be further modified for isolating different subtypes of ECs from the human heart tissue.

Graphical Abstract

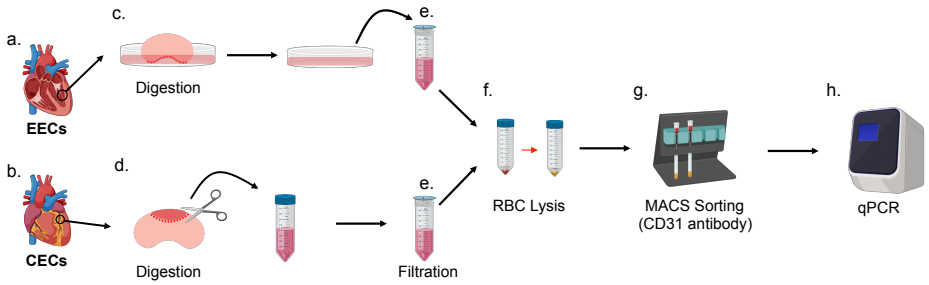

Figure Caption: Diagram of digestion set up of the CECs and EECs, and following arrangement of cell sorting. (a) Innermost free ventricular wall and (b) outermost ventricular free wall were (c) immersed in digestion buffer or (d) chopped into small pieces and digested in digestion buffer respectively. (e) Collection and filtration of cell solutions following with (f) RBC lysis and (g) MACS sorting using CD31 antibody. (h) Purified ECs were processed for qPCR verification. RBC: red blood cells; MACS: Magnetic-activated cell sorting.

Summary
Successful isolation of EECs and CECs is necessary to achieve comprehensive knowledge of these two cell populations, which can be utilized in both the research and clinical setting. Determining growth and differentiation factors of these cell populations would provide a reference for the differentiation of endothelial subtypes from induced pluripotent stem cells. Further, complete identification of the variances in the development, regulation, and function of EECs and CECs is vital for understanding the genomic and epigenomic factors responsible for numerous heart diseases in a cell-type specific manner. Because of the importance and broad opportunity for utilization of our protocol, we believe that it would be of value to JoVE readers.

Submission Invitation
9/30/2019: Received publication inquiry from Dr. Kyle Jewhurst.
10/01/2019: Received invitation to submit a manuscript to JoVE from the editorial.

Suggest Reviewers
We would like to suggest the following investigators with strong expertise in the fields of endothelial biology and heart development, as potential reviewers for our study.

Zhen Chen, Assistant Professor, Department of Diabetes Complications and Metabolism, City of Hope National Medical Center, zhenchen@coh.org;

Sang Ging Ong, Assistant Professor, Pharmacology and Medicine, University of Illinois Chicago, sangging@uic.edu

Shijun Hu, Professor and Deputy Director, Institute for Cardiovascular Science, Soochow University, shijunhu@suda.edu.cn

Jason Yuan, Professor, Section of Physiology, University of California San Diego, jxyuan@ucsd.edu

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John Shyy, Professor, Institute of Engineering in Medicine, University of California San Diego, jshyy@ucsd.edu

You have our assurance that all co-authors have read and are in agreement with the final submitted version of the manuscript. This manuscript is not under consideration for publication elsewhere.

We are grateful for your time and consideration of this manuscript for potential publication in JoVE, and we look forward to your feedback.

Sincerely,

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