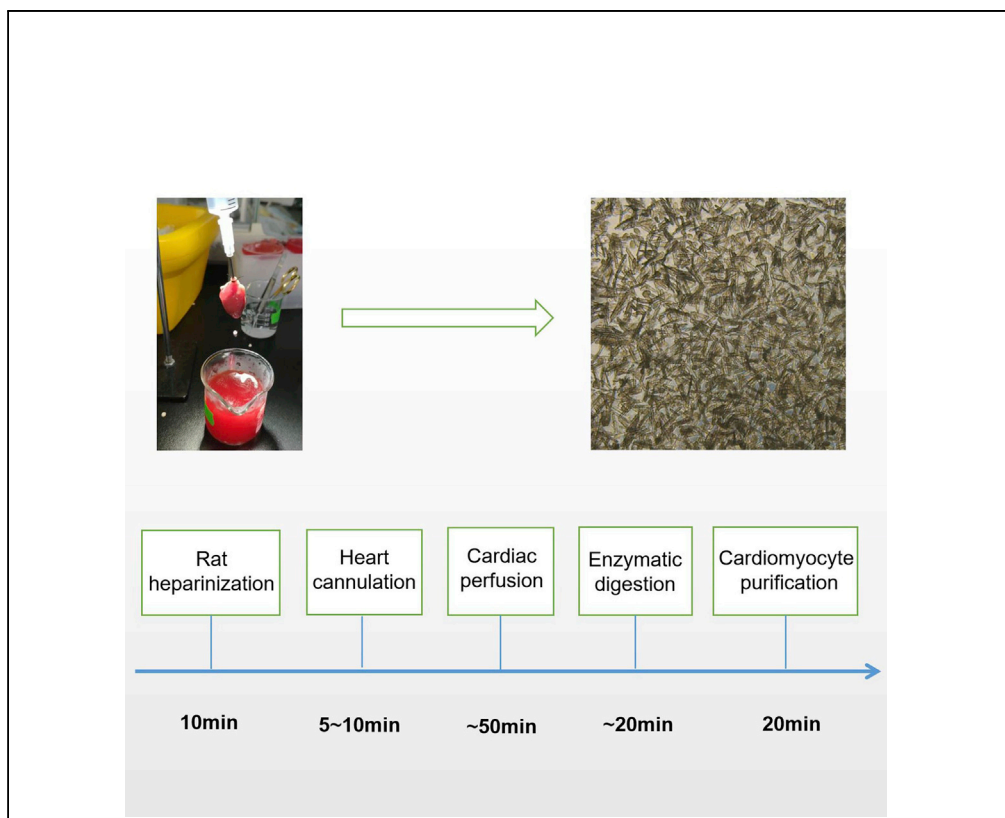


Protocol

Protocol for Isolation of Viable Adult Rat Cardiomyocytes with High Yield



Isolation of high-quantity and high-quality ventricular cardiomyocytes from adult rats is critical to study heart physiology and pathology and for drug toxicity screening. It remains challenging to produce a high yield of viable cardiomyocytes from rats. Here, we present our modified enzymatic digestion protocol that relies on the Langendorff device to generate large numbers of viable cardiomyocytes consistently. The most critical parts of this protocol are the selection of rat age and digestion time to obtain viable cardiomyocytes.

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HIGHLIGHTS

The age of the rat is critical for the perfusion digest to obtain viable cardiomyocytes

Blebbistatin is vital for the isolation and culture of adult cardiomyocytes

Protease treatment increases the yield of cardiomyocyte

Dead cells and other cell types are separated by gravity

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Protocol

Protocol for Isolation of Viable Adult Rat Cardiomyocytes with High Yield

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SUMMARY

Isolation of high-quantity and high-quality ventricular cardiomyocytes from adult rats is critical to study heart physiology and pathology and for drug toxicity screening. It remains challenging to produce a high yield of viable cardiomyocytes from rats. Here, we present our modified enzymatic digestion protocol that relies on the Langendorff device to generate large numbers of viable cardiomyocytes consistently. The most critical parts of this protocol are the selection of rat age and digestion time to obtain viable cardiomyocytes.

For complete details on the use and execution of this protocol, please refer to Liu et al. (2019) and Qin et al. (2020).

BEFORE YOU BEGIN

Prepare the solutions before you start the adult cardiomyocytes isolation. Refer to [Key Resources Table](#) and [Materials and Equipment](#) sections for a complete list of materials and equipment.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Heparin	Sigma-Aldrich	Cat# H3393
Sodium pentobarbital	Sigma-Aldrich	Cat# P3761
Type II collagenase	Biochemical Corporation	Cat# LS004176
Hyaluronidase	Sigma-Aldrich	Cat# H3506
Blebbistatin	TargetMol	Cat# T6038
Bovine Serum Albumin	Sigma-Aldrich	Cat# V900933
0.25% trypsin-EDTA	Gibco	Cat# 25200056

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
NaCl	Sigma-Aldrich	Cat# V900058
KCl	Sigma-Aldrich	Cat# V900068
KH ₂ PO ₄	Sigma-Aldrich	Cat# V900041
D-glucose	Sigma-Aldrich	Cat# G8270
HEPES	Sigma-Aldrich	Cat# V900477
CaCl ₂	Sigma-Aldrich	Cat# V900266
Mg-ATP	Sigma-Aldrich	Cat# A9187
NaH ₂ PO ₄	Sigma-Aldrich	Cat# V900060
M199	Sigma-Aldrich	Cat# M2520
NaHCO ₃	Sigma-Aldrich	Cat# V900182
Glutathione	Sigma-Aldrich	Cat# G6013
Creatine	Sigma-Aldrich	Cat# C3630
L-carnitine	Sigma-Aldrich	Cat# C0158
taurine	Sigma-Aldrich	Cat# T8691
Insulin-transferrin-selenium-X	Thermo Fisher Scientific	Cat# 51500056
Fetal bovine serum	Thermo Fisher Scientific	Cat# 12483020
Pen/Strep(100×)	Thermo Fisher Scientific	Cat# 10378016
Laminin	Thermo Fisher Scientific	Cat# 23017015
Software and Algorithms		
Image J	NIH	https://imagej.nih.gov/ij/download.html
zen	Zeiss	https://www.zeiss.com/microscopy/int/software-cameras.html
Experimental Models: Organisms/Strains		
Rat	Shanghai SLAC	Cat# SlacSD
Other		
50 mL/100 mL beakers	Sinoreagent	Cat# 91110104/91110105
15 mL and 50 mL Centrifuge tube	Thermo Fisher Scientific	Cat# 339650/339651
35 mm petri dish	Thermo Fisher Scientific	Cat# 121V
Surgical silk	Jianhuan Medical	Cat# A221
10 mL Syringe	Thermo Fisher Scientific	Cat# S7510-10
18G flat end syringe needle cannula with graves	BD	Cat# 302032
Fine-tip forceps	Jianhuan Medical	Cat# 47000500
Scissors	Sangon Biotech	Cat# F519232
Langendorff apparatus	Double baker warming coil from Hazvard Apparatus	Cat# 508382
Peristaltic pump	Easypump	Cat# YZ1515x
Water bath	Shanghai Yiheng	MP-5H
Iron stand	Saieise	K124001
Inverted microscope	Leica	DMI8
Refrigerated centrifuge	Hettich	Universal 320R0

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Guohua Gong (guohgong@tongji.edu.cn).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

This study did not generate any unique datasets or code.

MATERIALS AND EQUIPMENT

Solution Preparation

Note: Prepare all solutions using sterilized demineralized, ultrapure water (dd H₂O). 18.2 Ω molecular biology grade H₂O is highly recommended. All solutions for the isolation should be freshly prepared from the Ca²⁺ free Krebs-Henseleit buffer (KHB) solution. All procedures are performed with standard aseptic technique.

Note: if reagents from alternative suppliers are used, you must validate the efficiency of cardiomyocyte isolation for the first time.

- Rat Cardiomyocyte Isolation Buffer (Ca²⁺-Free KHB, pH 7.4), 2 L

Reagent	Final Concentration (mM)	Volume (mL)
NaCl (1 M)	118	236
KCl (0.5 M)	4.7	18.8
HEPES (1 M)	10	20
KH ₂ PO ₄ (0.25 M)	0.6	4.8
NaH ₂ PO ₄ (0.25 M)	0.6	4.8
MgSO ₄ ·7H ₂ O (100 mM)	1.2	24
Glucose (1 M)	11	22
ddH ₂ O	n/a	1669.6
Total	n/a	2000

Note: The solution needs to be sterilized through the filter. It can only be stored for two weeks at 4°C.

- Rat Cardiomyocyte Isolation Buffer (Ca²⁺ KHB): Add 10 mL CaCl₂ (100 mM stock) to 1 L Ca²⁺-Free KHB solution.
- Trypsin-ETDA solution: Dilute 0.25% trypsin-EDTA with 0.01 M PBS to a final concentration of 0.05%.
- Stop Buffer: Add 1 g BSA and 5 μl 100 mM Blebbistatin to Ca²⁺ KHB buffer.
- 1000 U/mL Heparin sodium solution: Dissolve 34.72 mg heparin sodium salt (180 U/mg) in 5 mL dd H₂O and store at 4°C.
- 80 mg/mL Pentobarbital solution: Dissolve 400 mg sodium pentobarbital in 5 mL dd H₂O and store at 4°C.
- Rat Collagenase Buffer (4×)

Reagent	Final Concentration	Amount
collagenase type II	472 U/mL	9440 U
hyaluronidase	0.9 mg/mL	18 mg
Blebbistatin (100 mM)	50 μ M	10 μ L
20 mL Ca ²⁺ -Free KHB buffer	n/a	20 mL
Total	n/a	~20 mL

Note: Most protocols use 2,3-butanedione monoxime to inhibit myocyte contraction, but we use the inhibitor Blebbistatin to stop myocyte contraction (Plackić and Kockskämper, 2018). It has been reported that Blebbistatin can extend the cultural life of adult cardiac myocytes (Kabaeva et al., 2008).

Note: Hyaluronidase cleaves hyaluronic acid, the main component of the extracellular matrix that links protein filaments, collagen fibers, and connective tissue cells, which can improve the osmotic ability of collagenase buffer in the cardiac tissue and thus dissociate more myocytes from cardiac tissue.

Note: The working concentration of the collagenase buffer is 1 \times (through adding 20 mL 4 \times collagenase buffer into 60 mL Ca²⁺-free KHB buffer in the container). Because the enzymes are mixtures, a different batch does not act identically. At first, we recommend purchasing small amount of collagenase type II. After the efficiency of digestion is verified, then purchase large quantity enzyme of the same batch. Collagenase type II from Worthington is a popular choice for cardiomyocyte isolation because it contains higher clostripain activity than other companies (Roth et al., 2014; Louch et al., 2011).

- M199 Medium

Reagent	Final Concentration	Amount
M199	n/a	1 bag (9.5 g)
NaHCO ₃	~2.2 g/L	~2.2 g
Glutathione	10 mM	3.073 g
BSA	0.2 g/L	0.2 g
ddH ₂ O	n/a	~1000 mL
Total	n/a	1000 mL

Note: The PH is adjusted to PH7.4 by NaHCO₃ and filtered.

- Culture Medium

Reagent	Final Concentration	Amount
M199 medium	n/a	93.89 mL
Pen/Strep(100 \times)	1 \times	1 mL
Creatine	5 mM	74.58 mg
L-carnitine	2 mM	32.24 mg
Taurine	5 mM	62.58 mg
Insulin-transferrin-selenium-X (100 \times)	0.1%	0.1 mL
FBS	5%	5 mL

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Reagent	Final Concentration	Amount
Blebbistatin (100 mM)	10 μ M	10 μ L
Total	n/a	100 mL

Note: Warm the medium to room temperature (23–26°C) before use.

STEP-BY-STEP METHOD DETAILS

Langendorff System Preparation

⌚ **Timing:** 30 min

⚠ **CRITICAL:** Pre-warm and oxygenate the perfusion solutions.

1. Before isolation, we need to fill up the Langendorff apparatus (Figure 1) (Liu et al., 2019), including circulating pipes, with 75% ethanol and soak for 1–2 h.

Note: For the first use, the Langendorff device should be soaked with 25% bleach for at least 12 h to sterilize the system.

2. Switch on the water bath to warm up the water bath-jacketed tubes of the Langendorff system to keep the circulating perfusion solution at 37°C into the heart.

Note: The temperature of the perfusate should also be checked regularly (Plačkić and Kockskämper, 2018).

3. Rinse the system 3 times with sterilized ddH₂O, and empty the system after each wash.
4. Fill Ca²⁺ KHB (~80 mL) and Ca²⁺-free KHB (~80 mL) into their containers (Figure 1).
5. Turn on the oxygen to oxygenate all solutions that are going to be used for perfusion to maintain sufficient O₂ supply.
6. Put all the surgical tools in a beaker with 75 % ethanol, and prepare heparin sodium solution and sodium pentobarbital solution.
7. Fill a 60 mm petri dish with Ca²⁺ KHB, and place it on ice.
8. Aspirate an appropriate amount (3–4 mL) of Ca²⁺ KHB to a 5 mL syringe, which connects to the cannula, with care to avoid introducing air bubbles.
9. Fix the 5 mL syringe on the iron stand, adjust the height so that the end of the cannula is inside the 60 mm petri dish, and then tie a surgical silk to the connecting site of the syringe for tying aorta.
10. Hold 50 mL of Ca²⁺ KHB with a small beaker on ice.

⏸ **Pause Point:** The system is ready for use and is fine for a break up to 4 h.

Surgery

⌚ **Timing:** 30 min

⚠ **CRITICAL:** Use ice-cold solutions, cannulate the heart on ice to reduce heart beating. Appropriately cannulate the aorta.

11. Heparinize rat (200–350 g) with sodium heparin intraperitoneally injected at 1000 U/kg to prevent the blood coagulation and the heart coronary embolism.

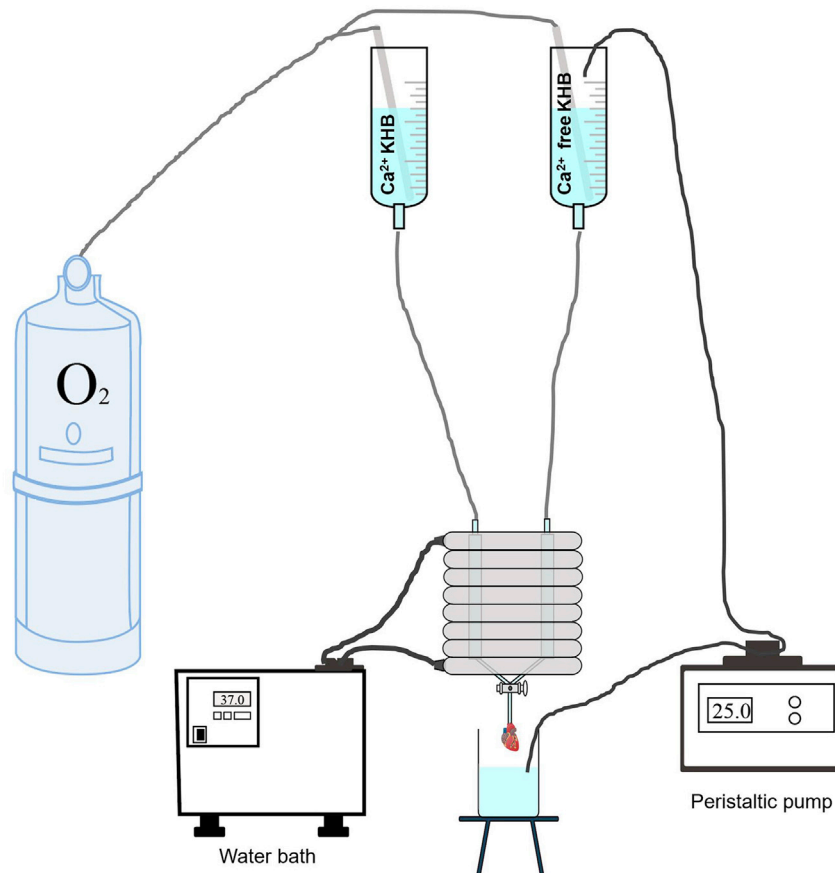


Figure 1. The Schematic Diagram of the Langendorff Perfusion System for Cardiomyocytes Isolation

Note: The weight of rat (correlated with age, generally, a 200–350 g rat is 6–8 weeks old) is crucial for the quality of the cardiomyocyte preparations. Less than 4 months old rat can be used for isolation.

Note: Heparin as an anticoagulant can be used *in vivo* or *in vitro*. In some protocols, heparin has been added to the cannulation solution for use (Plaćić and Kockskämper, 2018). However, instead of using heparin in the cannulation solution, we injected heparin before harvesting the heart, which can let the anticoagulant blood flow into the coronary to prevent the formation of embolism.

12. After 10 min, anesthetize the rat with sodium pentobarbital intraperitoneally injected at 80 mg/kg.
13. When the rat is in deep sleep (~5 min after injection and no response to toe pinch), spray chest and upper abdomen with 75 % ethanol, make a large incision on chest wall, cut through ribs on both sides to fully expose heart and lung.
14. Quickly remove the heart with the thymus and intact aortic arch. Removing the heart and some lung tissues together is better for saving time.
15. Put the heart into the small beaker with ice-cold Ca^{2+} KHB, gently press the heart against beaker wall 2–3 times to squeeze blood out, transfer the heart to the 60 mm petri dish with ice-cold Ca^{2+} KHB.

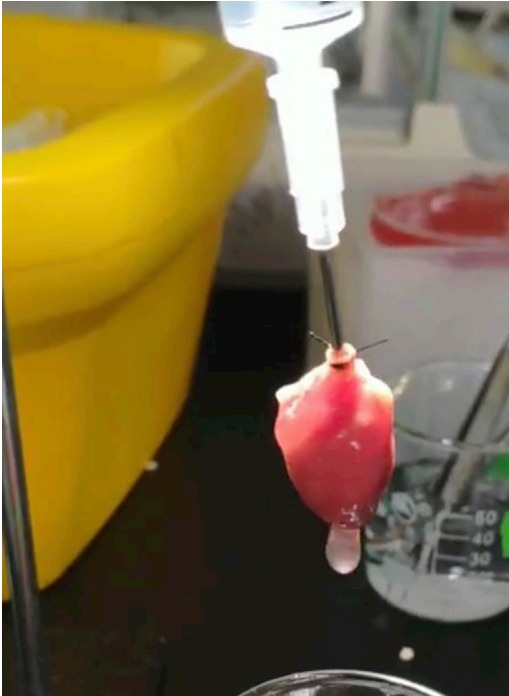


Figure 2. Cannulation of the Heart

16. Carefully remove other tissues and clean up the aortic root (leave 3–5 mm long of the aorta) with forceps and scissors.
17. Cannulate the heart with the cannula, and ligate twice with the prepared surgical silk to ensure no solution leaks from the ligating site (Liu et al., 2019).

△ **CRITICAL:** Cannulating the heart quickly is crucial for the quality of the cardiomyocyte isolation. During the isolation procedure, the cannula should not be inserted too deeply into the aorta, as this could lead to mechanical damage of the aortic valve leaflets and cause inefficient perfusion and poor digestion of the heart. The total time from the heart is removed to mounted and perfused on the Langendorff system should be as short as possible to avoid cell damage caused by ischemia and hypoxia (Plackić and Kockskämper, 2018). We recommend a time of less than 10 min and usually perform these steps within 5 min.

18. Slowly inject Ca^{2+} KHB into the aorta to exclude blood from the coronary artery and the heart cavity (Figure 2), and quickly hang the heart on the Langendorff apparatus.

Note: The cannula with the heart must be carefully connected to the Langendorff system to avoid introducing air bubbles.

Heart Perfusion

⌚ **Timing:** 50 min

△ **CRITICAL:** Ensure sufficient perfusion to dissociate cells from the heart tissue.

19. Perfuse Ca^{2+} KHB for 5 min at high flow rate (1–2 drops/s) (Figure 3A).

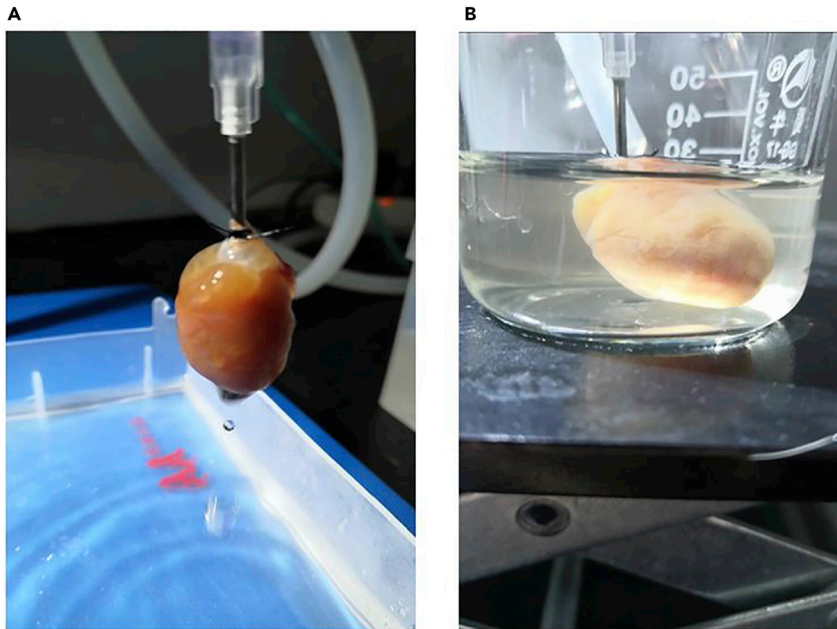


Figure 3. Perfused Digestion of the Heart

- (A) The rat heart is perfused with the KHB solution.
(B) Collagenase solution perfused heart.

Note: Enough Ca^{2+} KHB perfusion time leads to better myocardial cell protection.

20. Switch to perfuse Ca^{2+} -free KHB for 4–5 min at the high flow rate. The heart stops beating in 1 min.
21. When there is around 60 mL Ca^{2+} -free KHB left in the container, add 20 mL 4× Rat Collagenase Buffer to the Ca^{2+} -Free KHB container to mix with the Ca^{2+} -Free KHB to get a total 80 mL 1× collagenase buffer in the whole perfusion system. Set a 40 min timer.

Note: Fill 60 mL solution into the Ca^{2+} -Free KHB container and mark the solution level on the container when you use it the first time.

22. Put the heart in a small beaker (50 mL), set up a tube to recirculate Collagenase buffer (set up the peristaltic pump at a speed of ~180 mL/min) (Figure 3B).
23. After 5 min, add 80 μl CaCl_2 (100 mM stock) into the beaker twice at 0.5–1 min interval (the final concentration of Ca^{2+} is 0.2 mM in 80 mL 1× collagenase buffer).

Note: We found that a higher level of calcium ions stimulated the heart to continuously beating during digestion, which would cause the quality of isolated cardiomyocytes to decline.

24. Monitor heart digestion: use the forceps to check the heart after the heart becomes swelling and white. Stop perfusion if the collagenase solution drops become quicker, and frequency reaches to 180–200 drops/min. In our experience, this process usually takes 35 min.

Note: The duration of the collagenase digestion varies and could last any time between 25 and 50 min. It depends on several factors, including weight, age, and strain of rat or disease model (cardiac remodeling)

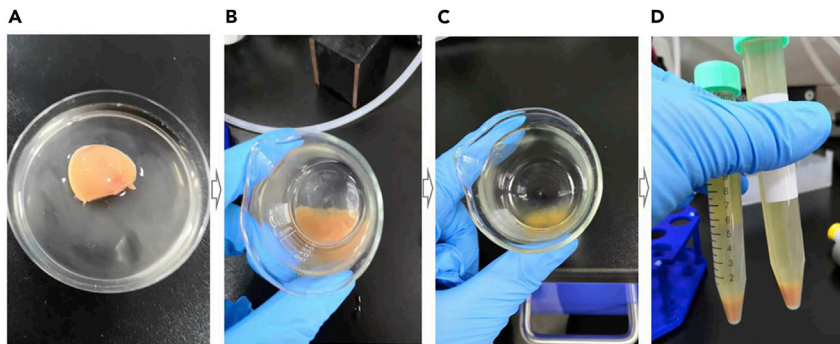


Figure 4. Enzyme Further Digests Cardiac Tissue

(A) The digested heart was cut off the atrium and aorta.
 (B and C) Myocardial tissue has been digested one time with enzyme (B); myocardial tissue has been digested three times with enzyme (C).
 (D) Collected cardiomyocytes in tubes.

Enzymatic Digestion (0.05% Trypsin-EDTA Solution)

⌚ Timing: 20 min

25. Take off perfused heart, and remove atrium and aorta, put the ventricular tissues in a small beaker with 10–15 mL enzyme solution (0.05% Trypsin-EDTA), and tear the ventricular tissue into small pieces with two fine-tip forceps (Figure 4A).
26. Pipette the tissue gently with 1 mL pipette (cut tip), and put in an incubator at 37°C for 5 min.
27. After 5 min, pipette the tissue again (Figure 4B), take one drop tissue solution to check the digest efficiency and cell viability under a microscope, stop the digestion if >80% rod shape cells are present individually (Figure 5A).
28. Allow the tissues to settle by gravity for 1 min, collect the supernatant from the beaker, and transfer to two or three 15 mL conical tubes so that each tube contains 5 mL of supernatant.
29. Add 5 mL Stop Buffer to the tube containing the 5 mL of supernatant, and pipette up and down 3 times to mix.
30. Add 10–15 mL 0.05% Trypsin-EDTA solution to the left precipitated cardiac tissue in the beaker; repeat steps 26 to 29 (Figure 4C).
31. Collect the sediment of cardiomyocytes through gravity (pipette up and down to mix and wait for 3–4 min) (Figure 4D). This will partially remove other small cells such as fibroblasts and endothelial cells.

Note: Repeat digestion with trypsin 1–2 times to get more myocytes if necessary.

Cardiomyocyte Purification

⌚ Timing: 20 min

32. Sequentially add 25 μ l CaCl_2 (100 mM stock) to cells in the conical tubes (each containing 10 mL solution) 3 times at 5 min intervals, mix well, and the fourth time add 50 μ l CaCl_2 , and mix well.
33. Collect the precipitated live cardiomyocytes through 30 g, 30 s centrifugation, and discard the supernatant. The left fibroblasts and endothelial cells are removed from myocytes' sedimentation by centrifuge.

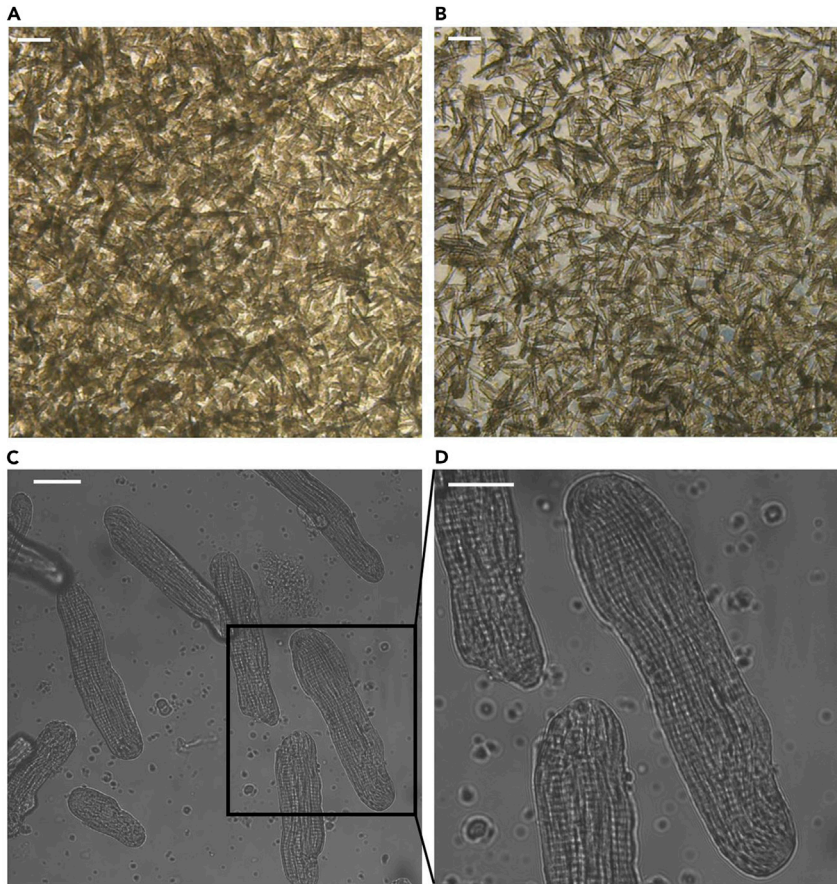


Figure 5. Purification and Culture of Cardiomyocytes

- (A) A representative image of cardiomyocytes collected from preliminary collagenase digestion of the cardiac. Scale bar, 100 μm .
 (B) A representative image of cardiomyocytes obtained from trypsin digestion. Scale bar, 100 μm .
 (C) A representative image of 1-day cultured cardiomyocytes. Scale bar, 30 μm .
 (D) Expanded image of cardiomyocytes. Scale bar, 20 μm .

34. Resuspend cells with 2 mL culture medium and merge all cells into one tube, then replenish the culture medium to 10 mL and mix well. Now, these cells are ready for experiments or culture (Figure 5B).

Pause Point: The cardiomyocytes in the medium can be incubated at 37°C, 5% CO₂ for 2–3 h.

Cardiomyocytes Culture

⌚ **Timing:** 40 min

35. Pre-coat the 6 well plates or dishes with 40 $\mu\text{g}/\text{mL}$ laminin (1 mL per well) for 30 min.

Note: This step should be done before cardiomyocyte purification for saving time.

36. Count the cell number and plate cells with 500 μl culture medium per well at a density of 5×10^4 cells/mL, then incubate at 37°C, 5% CO₂.

37. Two hours later, after cardiomyocytes attached to the plate, gently change the medium with 2 mL fresh culture medium for continued culture (Figures 5C and 5D).

EXPECTED OUTCOMES

In our protocol, we use collagenase and hyaluronidase to perfuse heart. Hyaluronidase cleaves hyaluronic acid, the main component of the extracellular matrix that links protein filaments, collagen fibers, and connective tissue cells, which can improve the osmotic ability of collagenase buffer in the cardiac tissue and thus dissociate more myocytes from cardiac tissue. A stronger proteinase, trypsin, is used to furtherly digest nonsingle cells (cell mass) and tissue after the perfused digestion, which can increase the yield of cardiomyocytes. At the same time, we use the cell contraction inhibitor Blebbistatin, which not only inhibits myocyte beating but also extends the cultural life of adult cardiac myocytes. Rapid and high yield viable cardiomyocytes were isolated from one adult rat heart through our protocol. It has been reported that approximately 3 million cardiomyocytes could be obtained from an adult rat heart (Campora et al., 2018). In our protocol, about 85% cells were rod shape in the digested cells, and $2.5\text{--}4.0 \times 10^6$ live cardiomyocytes could be derived from one heart. These cells are enough for seeding ten 6-well plates at a density of 3×10^4 cells/well. These cardiomyocytes can be cultured to 6–7 days with a medium change every 2 days (Liu et al., 2019).

LIMITATIONS

As stated above, the time required for cardiomyocyte isolation depends on weight, age, sex, and strain of rat or disease model; thus, it cannot be precisely defined here.

The lot-to-lot variability of protease activities from type 2 collagenase preparations is very high (Plackić and Kockskämper, 2018). Thus, the concentration of collagenase and digestion time needs to be modified if a new batch of collagenase is used.

The yield of rod-shaped cardiomyocytes from one adult rat heart may vary ($2.5\text{--}4.0 \times 10^6$ cells) with body weight, strain, and collagenase activities. (Li et al., 2014; Plackić and Kockskämper, 2018)

TROUBLESHOOTING

Problem 1

Low yield (less than 5×10^5 cells from one heart if the cardiac tissue has not been sufficiently digested) of Rod-shaped Cardiomyocytes

Potential Solutions

Some modifications can be taken, including increasing heparin to 1250 U/kg, reducing the time of heart cannulation to 3 min, increasing oxygen supply, using more excitation-contraction coupling inhibitor (up to 15 μM Blebbistatin) (Farman et al., 2007; Kabaeva et al., 2008). The sufficient perfusion time (up to 50 min) may be needed.

Problem 2

Isolated Cardiomyocytes with Low viability (the none rod shape cells are the majority)

Potential Solution

Slightly decrease one-fourth of the concentrations of collagenase and trypsin.

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AUTHOR CONTRIBUTIONS

G.-G.H. conceived, designed, and supervised the project. T.-X.G., G.-M., and L.-A.Q. conducted most of the experiments. L.-B.L. and J.-W.T. performed partial data analysis. Q.-Y. provided valuable suggestions. G.G. and T.-X.G. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Liu, B.L., Li, A.Q., Qin, Y., Tian, X.G., Gao, M., Jiang, W.T., and Gong, G.H. (2019). Comparative study on isolation and mitochondrial function of adult mouse and rat cardiomyocytes. *J. Mol. Cell. Cardiol.* *136*, 64–71.
- Qin, Y., Li, A.Q., Liu, B.L., Jiang, W.T., Gao, M., Tian, X.G., and Gong, G.H. (2020). Mitochondrial fusion mediated by fusion promotion and fission inhibition directs adult mouse heart function toward a different direction. *FASEB. J.* *34*, 663–675.
- Roth, G.M., Bader, D.M., and Pfaltzgraff, E.R. (2014). Isolation and physiological analysis of mouse cardiomyocytes. *J. Vis. Exp.* *91*, e51109.
- Campora, S., Alberte, P.S., Bruno, C., and Ghersi, G. (2018). Isolation of adult rat cardiomyocytes using recombinant collagenases. *Chem. Eng. Trans.* *64*, 25–30.
- Louch, W.E., Sheehan, K.A., and Wolska, B.M. (2011). Methods in cardiomyocyte isolation, culture, and gene transfer. *J. Mol. Cell. Cardiol.* *51*, 288–298.
- Plackić, J., and Kockskämper, J. (2018). Isolation of atrial and ventricular cardiomyocytes for in vitro studies. In *Experimental Models of Cardiovascular Diseases*, K. Ishikawa, ed. (Springer), pp. 39–54.
- Li, D.X., Wu, J., Bai, Y., Zhao, X.C., and Liu, L.J. (2014). Isolation and culture of adult mouse cardiomyocytes for cell signaling and in vitro cardiac hypertrophy. *J. Vis. Exp.* *87*, e51357.
- Farman, G.P., Tachampa, K., Mateja, R., and Cazorla, O. (2007). Blebbistatin: use as an inhibitor of muscle contraction. *Pflug. Arch. Eur. J. Phys.* *455*, 995–1005.
- Kabaeva, Z., Zhao, Mei., and Michele, D.E. (2008). Blebbistatin extends the culture life of adult mouse cardiac myocytes and allows efficient and stable transgene expression. *Am. J. Physiol. Heart Circ. Physiol.* *294*, H1667–H1674.