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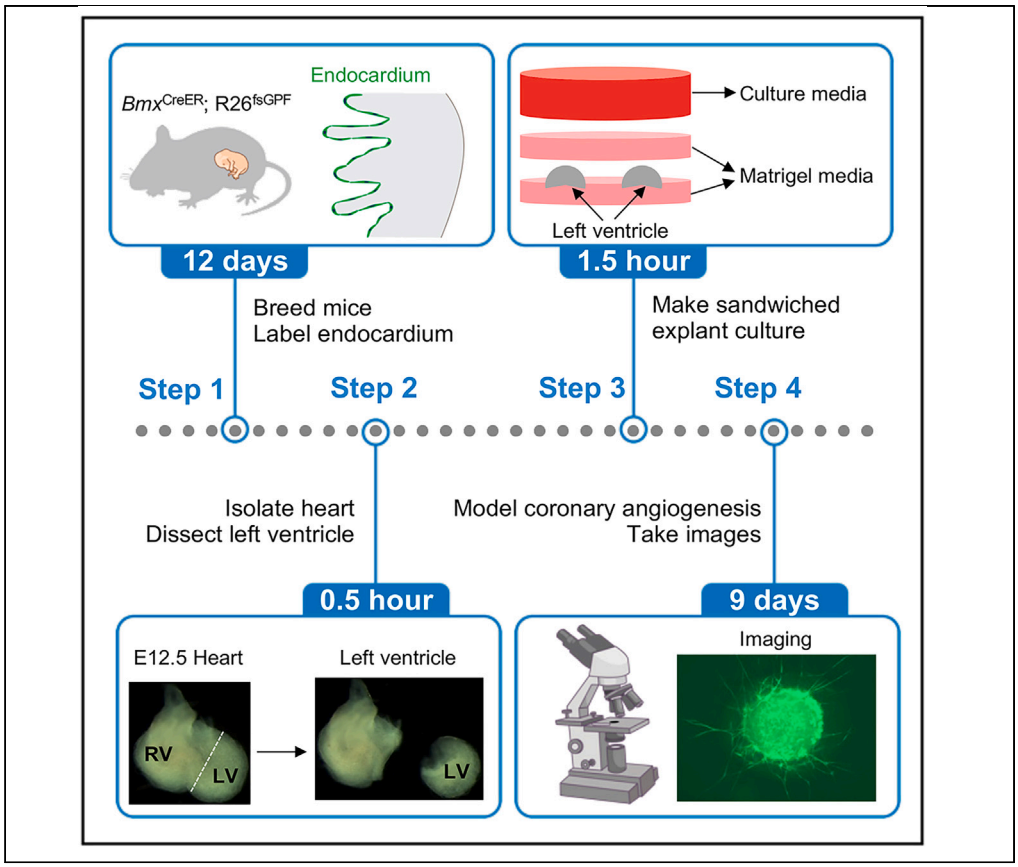
Protocol

A sandwiched ventricular explant assay to model mouse coronary angiogenesis *ex vivo*



Developing an *ex vivo* system that mimics *in vivo* developmental coronary angiogenesis provides an improved understanding of its underlying molecular and cellular mechanisms. Here, we present a sandwiched embryonic ventricular explant assay to model mouse coronary angiogenesis *ex vivo*. We describe steps for breeding mice, labeling endocardial cells, isolating murine hearts, dissecting left ventricles, and making sandwiched explants in Matrigel. We then detail procedures for modeling coronary angiogenesis and taking images.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Protocol for modeling developmental coronary angiogenesis *ex vivo*

Steps for dissecting the left ventricle from a mouse embryo

Details on how to make the sandwiched ventricular explant culture

Screening protocol for potential factors regulating coronary angiogenesis

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A sandwiched ventricular explant assay to model mouse coronary angiogenesis *ex vivo*

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SUMMARY

Developing an *ex vivo* system that mimics *in vivo* developmental coronary angiogenesis provides an improved understanding of its underlying molecular and cellular mechanisms. Here, we present a sandwiched embryonic ventricular explant assay to model mouse coronary angiogenesis *ex vivo*. We describe steps for breeding mice, labeling endocardial cells, isolating murine hearts, dissecting left ventricles, and making sandwiched explants in Matrigel. We then detail procedures for modeling coronary angiogenesis and taking images.

For complete details on the use and execution of this protocol, please refer to Lu et al. (2023)[1]

BEFORE YOU BEGIN

Institutional permissions

Mice are used in this protocol. All mouse experiments and procedures are performed according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC) of Albert Einstein College of Medicine.

Prepare the ventricular endocardial labeled time-pregnant mice

Timing: 12 days

The protocol below describes specific steps for Bmx^{CreER};$R26^{fsGFP}$ mice. However, this protocol also works with $Nfatc1^{Cre}$;$R26^{fsGFP}$ and $Npr3^{CreERT2}$;$R26^{fsGFP}$ mice. Bmx^{CreER}, $Nfatc1^{Cre}$ and $Npr3^{CreERT2}$ are Cre deleter mice used for genetic deletions in the cardiac endocardial cells.

1. Breed the Bmx^{CreER} mice with $R26^{fsGF}$ and reporter mice and check the vaginal plug in the next morning.

   Note: Noontime on the day of vaginal plugs detection is designated as E0.5.

2. Administer Tamoxifen (100 mg/kg) to pregnant female mice by oral gavage, to induce reporter gene expression and label ventricular endocardial cells at E11.5, one day before collecting the embryos at E12.5.

   Note: We present the procedure for labeling the endocardial cells in Bmx^{CreER};$R26^{fsGFP}$ mice at E11.5, to study the second wave of developmental coronary angiogenesis, by the

ventricular endocardial cells, for the formation of the trabecular coronary vessels. We have also labeled this line at E9.5 or E10.5, which allows to study the first wave of developmental coronary angiogenesis by the ventricular endocardial cells, for the formation of the coronary vessels at the compact myocardium.[2]

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Recombinant VEGFA120	R&D	Cat# 494-VE-025
Recombinant TGFB2	R&D	Cat# 7346-B2-005
Endothelial Cell Growth Basal Medium	R&D	Cat# CCM029
Endothelial Cell Growth Supplement without VEGF (50×)	R&D	Cat# 390634
MEM Non-essential Amino Acid Solution (100×)	Sigma	Cat# M7145
ITS Liquid Media Supplement (100×)	Sigma	Cat# I3146
L-Glutamine solution (100×)	Sigma	Cat#G7513
Penicillin/Streptomycin (100×)	Sigma	Cat# 516106
Tamoxifen	Sigma	Cat# T5648-1G
Matrigel® Growth Factor Reduced	Corning	Cat# 354230
DPBS		Cat# pap
Experimental models: Organisms/strains		
Bmx^{CreER}	Ehling et al.[3]	N/A
R26^{fsGFP}	Miyoshi et al.[4]	N/A
Other		
Scissors	Fine Science Tools	Cat#14084-09
Forceps	Fine Science Tools	Cat#11253-20
Transfer pipettes	Thermo	Cat#202-1SPK
Nikon dissection microscope	Nikon	C-BD115
Carl Zeiss fluorescent microscope	Zeiss	Axio Observer A1

MATERIALS AND EQUIPMENT

Ventricular explant culture Basal Media

Reagent	Final concentration	Amount
Endothelial Cell Growth Basal Medium	N/A	9.4 mL
Endothelial Cell Growth Supplement without VEGF (50×)	1×	200 μL
MEM Non-essential Amino Acid Solution (100×)	1×	100 μL
ITS Liquid Media Supplement (100×)	1×	100 μL
L-Glutamine solution (100×)	1×	100 μL
Penicillin/Streptomycin (100×)	1×	100 μL
Total	**N/A**	**10 mL**

Store at 4°C. Preferably use within one week.

Matrigel media

Mix ventricular explant culture Basal Media with Matrigel® Growth Factor Reduced at 1:1 on ice.

Right before use.

Ventricular explant culture conditional media 1

Ventricular explant culture Basal Media supplemented with TGFb2 (final concentration: 2 ng/mL).

Store at 4°C. Preferably use within one week.

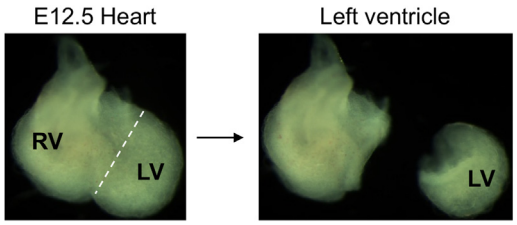

Figure 1. Dissecting the left ventricle
RV: right ventricle; LV: left ventricle.

Ventricular explant culture conditional media 2

Ventricular explant culture Basal Media supplemented with TGFb2 (final concentration: 10 ng/mL).

Store at 4°C. Preferably use within one week.

Ventricular explant culture conditional media 3

Ventricular explant culture Basal Media supplemented with VegfA120 (final concentration: 10 ng/mL).

Store at 4°C. Preferably use within one week.

STEP-BY-STEP METHOD DETAILS

Dissect the left ventricle

Timing: 30 min

E12.5 left ventricles are collected under a dissection microscope.

1. E12.5 *Bmx*^{CreER};*R26*^{fsGFP} mouse embryos are collected from the pregnant female mice which were sacrificed immediately after anesthesia after inhaling isoflurane, and their hearts are isolated using autoclaved scissors and forceps and collected in cold DPBS.

   Note: No perfusion is necessary as we collected the embryonic ventricle for culture.

2. Check the hearts' GFP expression under a fluorescent microscope, the GFP positive hearts are *Bmx*^{CreER};*R26*^{fsGFP} and the endocardial cells are labeled by GFP. The subsequent experiments are using these GFP positive hearts.
3. Dissect out the left ventricle from the heart using a fine forceps as shown in Figure 1.

   Note: The whole heart was in the cold DPBS in 60mm Petri dishes.

   a. use one forceps to hold the heart on the bottom of the Petri dishes.
   b. use another forceps to dissect the left ventricle as shown in Figure 1.
   c. put the left ventricles in cold DPBS on ice.

   Note: Please don't too long time on ice, we usually put on ice for no longer than 2 hours.

Make sandwiched left ventricular explant culture

Timing: 9 days

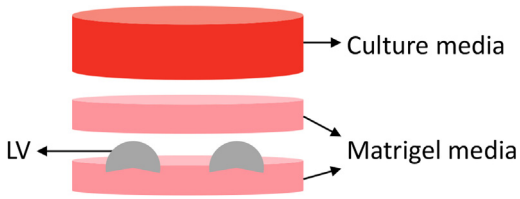

Figure 2. Make sandwiched left ventricular explant culture

Left ventricular explants are sandwiched in the Matrigel media and cultured within the Matrigel media as shown in Figure 2.

4. Make the Matrigel media freshly on ice, add 150 μL/well Matrigel media to a 4-well dish

   Note: Please use the 1000 μL precooled (4°C) pipette to add Matrigel media.

   Note: Please make 300 μL/well Matrigel media here, use 150 μL/well this step and the remaining 150 μl/well Matrigel media was kept on ice and will be used in the 7th step.

5. Put the Matrigel media-coated 4-well dish in 37°C 5% CO_2 cell culture incubator for 25–30 min to allow the Matrigel polymerized (solidified).

   ⚠ CRITICAL: The incubation time is key and needs to be optimized for good ventricular explant culture. Too short incubation time will lead the ventricle to attach to the dish bottom and too long time will lead the ventricle to poorly growth on the Matrigel media.

   Note: If one can finish the dissection step within 25 min, one can make the Matrigel media first, and then do the dissection step during Matrigel media incubation time. Otherwise, do the dissection step first (put the left ventricle on the ice) and then make the Matrigel media incubation.

6. Pipette 1 to 3 left ventricles in DPBS using a 3 mL transfer pipette and put them on one well of a Matrigel media coated 4-well dish. Allow the cutting side of left ventricles facing the bottom and gently remove the extra DPBS using a 20 μl pipette

   Note: We usually culture one to three ventricles/well in the 4-well dish.

7. Add slowly another 150 μL Matrigel media per well and incubate for 30 min in 37°C 5% CO_2 cell culture incubator to let the Matrigel solidified.
8. Add 200 μL/well ventricular explant culture Basal Media supplemented with 2 ng/mL TGFb2 (Conditional Media 1) and use ventricular explant culture Basal Media without TGFb2 as control. Culture the sandwiched left ventricles in 37°C 5% CO_2 cell culture incubator for one day and today is day 0. On day 1 and day 2, carefully remove whole conditional media 1 with 100 μL pipette and change to fresh conditional media 1 for two days.

   Alternatives: We can add 200 μL/well ventricular explant culture Basal Media supplemented with 10 ng/mL TGFb2 (Conditional Media 2) and use ventricular explant culture Basal Media without TGFb2 as control. Culture the sandwiched left ventricles in a 37°C 5% CO_2 cell culture incubator for one day and today is the day 0. On day 1 and day 2, carefully remove whole conditional media 2 with 100 μL pipette and change to fresh Basal Media for two days.

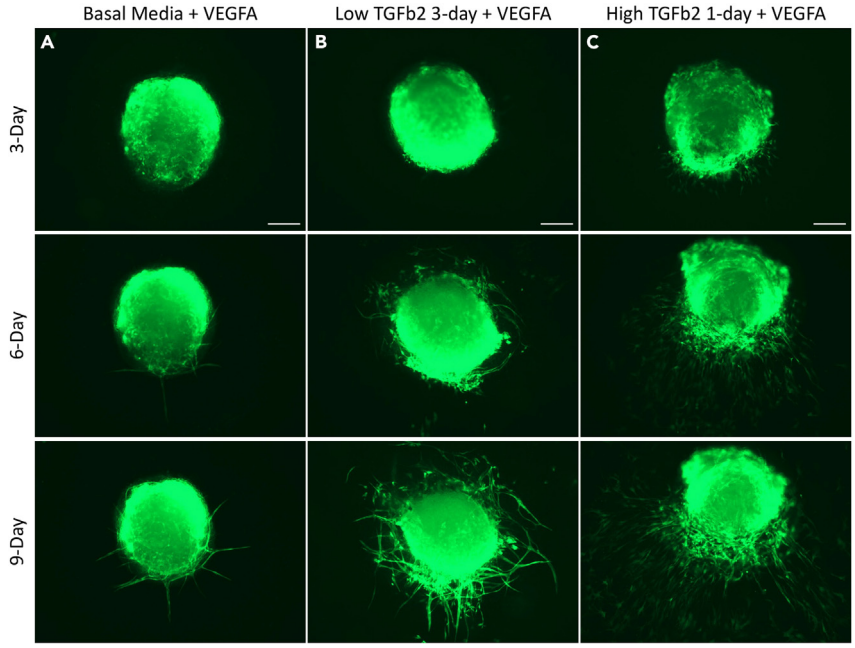

Figure 3. Images of coronary angiogenesis during left ventricular explant culture

(A–C) Show angiogenic sprouting by GFP-expressing descendants from *Bmx*^{CreER} marked ventricular endocardial cells at E11.5 after basal treatment 3 days (A), 2 ng/mL TGFb2 treatment 3 days (B), or 10 ng/mL TGFb2 treatment for 1 day then basal treatment 2 days (C), then following subsequent VEGFA treatment from day 4–9 culture. Images were taken on 3-day (top panels), 6-day (middle panels), and 9-day (low panels). Scale bars: 100 μm.

9. On the day 3, switch to ventricular explant culture Basal Media supplemented with 10 ng/mL VEGF120 (Conditional media 3), carefully change the culture media every other day (change to fresh conditional media 3 on the day 5 and day 7) for another 6 days.

   Note: Take images of cultured left ventricles under the fluorescent microscope on day 3, 6, and 9 (Figure 3) to document coronary angiogenesis and formation of coronary networks.

EXPECTED OUTCOMES

A successful sandwiched ventricular explant culture will show the process of the coronary vascular network formation by the GFP-labeled coronary endothelial cells derived from the ventricular endocardial progenitors, a process that resembles developmental coronary angiogenesis *in vivo*. After 3 days of left ventricular explant culture with the Basal Media supplemented with TGFb2, there will be significant induced angiogenic sprouting by GFP-expressing descendants from the *Bmx*^{CreER} marked left ventricular endocardial cells. After another 3–6 days of ventricular explant culture with the Basal Media supplemented with VEGF120, there will be significantly enhanced coronary angiogenesis by GFP-expressing descendants from the *Bmx*^{CreER} marked left ventricular endocardial cells at E11.5, leading to the formation of GFP-expressing coronary vascular networks.

LIMITATIONS

Although this sandwiched ventricular explant assay is a powerful technique to model developmental coronary angiogenesis *ex vivo* and to explore the potential underlying molecular and signaling mechanisms, it has some limitations. One limitation is the developmental stage of the left ventricle. The left or whole ventricle of the embryonic heart from E10.5 to E12.5 can be used in this assay. The left or whole ventricle from embryos older than E13.5 may be not suitable for this explant culture assay as the older ventricle is thick and endocardial cells are hard to spout out and older endocardial

cells will gradually lose the potential of coronary angiogenesis. Another limitation is the culture media, which preferentially support endothelial cell growth and may be not optimal for other cell types (like cardiomyocytes). Coronary angiogenesis is a complex biological process requiring interactions between cardiomyocytes and endocardial/endothelial cells.

TROUBLESHOOTING

Problem 1

No coronary angiogenesis formation after left ventricular explant culture.

Potential solution

- Practice the whole experiment more times to master the technique and procedure (step 1 to step 3). The key to a good outcome is to dissect the left ventricle as gently and quickly as possible to avoid tissue damage and cell death.
- Make sure the slicing side of the left ventricle attaches to the bottom of the well coated by the Matrigel (step 6).

Problem 2

Low coronary angiogenesis formation after left ventricular explant culture.

Potential solution

- ake sure the concentration of growth factors (like TGFb2 and VEGF120) in the culture media is correct and functional (steps 8, 9).
- Make sure the embryonic stage (E12.5) is correct as older endocardial cells gradually lose the coronary angiogenesis potential (step 1).

Problem 3

Two or three left ventricular explant's coronary angiogenesis growth together.

Potential solution

Make sure ventricles in each well are distributed evenly (step 6), do not too close to each other. Alternatively, just put one left ventricle in the middle of each well.

Problem 4

Left ventricular explant floats during changing media.

Potential solution

Make sure the 7^{th} step incubation time is enough long (30 min, no less than 25 min) to let the Matrigel solidify, and also carefully change culture media (like using pipettes but not the suction pump to change media).

Problem 5

The color of culture media in the 4-well dish looks too yellow.

Potential solution

Make sure to make the culture media following the recipe and may change the Conditional Media daily (step 9). Alternatively, just culture one or two left ventricles in each well.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Bin Zhou (bin.zhou@einsteinmed.edu).

Materials availability

The materials used and generated in this study are available from the lead contact upon reasonable request with a completed Materials Transfer Agreement.

Data and code availability

This protocol does not generate any datasets or code.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

P.L. and B.Z. conceived the concept. P.L. and B.Z. designed the experiments. P.L., B.W., Y.W., and J.Z. conducted experiments. P.L. and B.Z. wrote the manuscript. B.Z. supervised experiments and provided grant support.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Lu, P., Wu, B., Wang, Y., Russell, M., Liu, Y., Bernard, D.J., Zheng, D., and Zhou, B. (2023). Prerequisite endocardial-mesenchymal transition for murine cardiac trabecular angiogenesis. Dev. Cell *58*, 791–805.e4. https://doi.org/10.1016/j.devcel.2023.03.009.

2. Wu, B., Zhang, Z., Lui, W., Chen, X., Wang, Y., Chamberlain, A.A., Moreno-Rodriguez, R.A., Markwald, R.R., O'Rourke, B.P., Sharp, D.J., et al. (2012). Endocardial cells form the coronary arteries by angiogenesis through myocardial-endocardial VEGF signaling. Cell *151*, 1083–1096. https://doi.org/10.1016/j.cell.2012.10.023.

3. Ehling, M., Adams, S., Benedito, R., and Adams, R.H. (2013). Notch controls retinal blood vessel maturation and quiescence. Development *140*, 3051–3061. https://doi.org/10.1242/dev.093351.

4. Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V.H., Butt, S.J.B., Battiste, J., Johnson, J.E., Machold, R.P., and Fishell, G. (2010). Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. J. Neurosci. *30*, 1582–1594. https://doi.org/10.1523/JNEUROSCI.4515-09.2010.