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Project Page Link: <https://review.jove.com/account/file-uploader?src=21792603>

Title: Generation and Phenotypic Characterization of a CRISPR/Cas9-Engineered *Cracd*-Deficient Mouse Model for Post-Myocardial Infarction Remodeling Studies

Authors and Affiliations:

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FINAL SCRIPT: APPROVED FOR FILMING



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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

Nikon SMZ745

2.5.1, 2.5.2, 2.6.1, 2.6.2, 2.6.3, 2.7.1

Videographer: Please film all SCOPE shots using the scope kit

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 27

Number of Shots: 60 (12 SCs)

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Shuang Zheng**: We study how CRACD deficiency influences heart remodeling and function after myocardial infarction, using a Cracd-deficient mouse model.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Shuang Zheng**: This protocol can be applied to study gene functions in post-MI cardiac remodeling by combining CRISPR knockout with Vevo strain imaging.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Weijiang Tan**: This protocol allows researchers to generate knockout mice and evaluate post-MI cardiac remodeling using echocardiography, Vevo strain, and histology.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll; 2.5.1.*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Shuang Zheng**: The biggest challenge while performing this protocol is establishing a consistent MI surgery and acquiring clear images for Vevo strain analysis.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.6.1*

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Weijiang Tan:** Using this procedure, researchers can also perform molecular studies, RNA-seq analysis, and long-term functional studies following this protocol.

- 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.6. **Shuang Zheng, Assistant Researcher, Guangdong Provincial Biotechnology Research Institute (Guangdong Provincial Laboratory Animals Monitoring Center, :** (authors will present their testimonial statements live)

- 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Weijiang Tan, Assistant Researcher, Guangdong Provincial Biotechnology Research Institute (Guangdong Provincial Laboratory Animals Monitoring Center):** (authors will present their testimonial statements live)

- 1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Guangdong Institute of Biotechnology

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Myocardial Infarction Model Establishment

Demonstrator: Weijiang Tan

- 2.1. To begin, use CRISPR/Cas9 (*Crispr-Cas-Nine*) gene editing technology to generate *Cracd* (*Krak-d*)-deficient mice on a C57BL/6N (*C-Fifty-Seven-B-L-Six-N*) background [1].
 - 2.1.1. Establishing shot of the talent with the generated *Cracd* deficient mice in the background.
- 2.2. Place the anesthetized mice in a supine position on a surgical platform [1-TXT] and confirm deep anesthesia by the absence of the pedal withdrawal reflex [2].
 - 2.2.1. Talent placing the mice in a supine position on a surgical platform. **TXT: Anesthesia: 3% Isoflurane**
 - 2.2.2. Talent confirming deep anesthesia by checking the pedal withdrawal reflex.
- 2.3. Then, perform orotracheal intubation using a 20-gauge intravenous catheter sheath [1]. Place a small tuft of cotton wool at the opening of the tube to confirm successful intubation as it moves rhythmically with the mouse's breathing [2]. Connect the tube to a mechanical ventilator upon successful intubation to maintain ventilation and anesthesia with 2 percent isoflurane [3]. **NOTE: The VO is edited for the additional shot**
 - 2.3.1. Talent performing orotracheal intubation using a 20-gauge intravenous catheter sheath. **NOTE: 2.3.1(1) and 2.3.1(2) represent two actions in a single shot**
Added shot: Talent places a small tuft of cotton wool was placed at the tube opening
 - 2.3.2. Talent connecting the tube to a mechanical ventilator.
- 2.4. Apply ophthalmic ointment to both eyes to prevent corneal drying [1]. Then, remove hair from the anterior chest using a depilatory cream [2], and disinfect the surgical area with povidone-iodine [3]. After fully exposing the skin, make a 1-to-1.5-centimeter incision in the left chest wall between the 2nd and 3rd intercostal spaces to open the thoracic cavity [4]. Then, incise the pericardium to fully expose the heart [5].
 - 2.4.1. Talent applying ophthalmic ointment to both eyes of the mouse.
 - 2.4.2. Talent removing hair from the anterior chest with depilatory cream.
 - 2.4.3. Talent disinfecting the surgical area with povidone-iodine.
 - 2.4.4. Talent making an incision in the left chest wall.
 - 2.4.5. Talent incising the pericardium to expose the heart. **NOTE: 2.4.5(1) and 2.4.5(2) represent two actions in a single shot**

2.5. Using the inferior border of the left auricle as a reference, pass a 7-0 atraumatic suture needle beneath the left anterior descending coronary artery, about 2 to 3 millimeters distal to its origin, to a depth of 0.5 to 1 millimeter [1] and tighten the ligature slowly to avoid myocardial tearing [2].

2.5.1. SCOPE: Talent passing a 7-0 atraumatic suture needle beneath the left anterior descending coronary artery to the required depth.

2.5.2. SCOPE: Talent tightens the ligature.

Videographer: Please film all SCOPE shots using the scope kit

2.6. Confirm successful occlusion by observing pallor of the left ventricular anterior wall distal to the ligation site within 30 seconds [1]. In sham-operated controls, open the chest [2] and pass the suture beneath the coronary artery without tying the knot [3].

2.6.1. SCOPE: Shot of the left ventricular anterior wall becoming pale distal to the ligation site.

2.6.2. SCOPE: Talent opening the chest of a sham-operated control mouse.

2.6.3. SCOPE: Talent passing the suture beneath the coronary artery without tying the knot.

2.7. After establishing the model, suture the ribs and skin to close the thoracic cavity [1]. Remove the endotracheal tube once the mouse resumes stable spontaneous breathing [2].

2.7.1. SCOPE: Talent suturing the ribs and skin with a 4-0 absorbable suture. **NOTE: 2.7.1(1) and 2.7.1(2) represent two actions in a single shot**

2.7.2. Talent removing the endotracheal tube from the mouse.

2.8. Place the mice on a heating pad at 37 degrees Celsius until fully conscious [1]. Administer postoperative analgesia according to Institutional Animal Care and Use Committee guidelines [2]. Closely monitor the postoperative health status for several days [3].

2.8.1. Talent placing the mice on a heating pad. **NOTE: 2.8.1 and 2.8.2 are filmed in a single shot**

2.8.2. Talent administering postoperative analgesia by subcutaneous injection.

2.8.3. Talent monitoring the postoperative condition of the mice.

2.9. ~~Record the number of mice that die within the first 7 days after myocardial infarction surgery [1].~~ **NOTE: Not filmed**

~~2.9.1. Talent recording mortality data for mice during the first 7 days after myocardial infarction surgery.~~

3. Echocardiographic Assessment

Demonstrator: Shuang Zheng

- 3.1. Power on the high-resolution small animal ultrasound imaging system and its integrated heated stage [1]. Ensure that the appropriate high-frequency transducer is securely connected to the mounting apparatus following the manufacturer's instructions [2]. Set the heated stage temperature to 37 degrees Celsius and allow it to stabilize [3].
 - 3.1.1. Talent powering on the high-resolution small animal ultrasound imaging system and heated stage.
 - 3.1.2. Talent securing the high-frequency transducer to the mounting apparatus.
 - 3.1.3. Talent setting the heated stage temperature to 37 degrees Celsius.
- 3.2. After anesthetizing the mouse until the pedal withdrawal reflex is absent [1-TXT], transfer the mice to the heated platform in a supine position [2]. Maintain anesthesia with 1 to 1.5 percent isoflurane delivered via a nose cone, adjusting to maintain a heart rate between 450 and 550 beats per minute [3].
 - 3.2.1. Talent checking the pedal reflex. **TXT: Anesthesia: 3% Isoflurane in O₂**
 - 3.2.2. Talent transferring an anesthetized mouse to the heated platform in a supine position. **NOTE: 3.2.2 and 3.2.3 are filmed in a single shot**
 - 3.2.3. Talent positioning the nose cone on the mouse for maintenance anesthesia.
- 3.3. Apply ophthalmic ointment to both eyes to prevent corneal drying [1]. Attach surface electrocardiogram electrodes to the limbs using electrode gel at the contact sites and secure them with tape [2]. Then, shave the thoracic region and apply the depilatory cream evenly for no more than 3 minutes [3]. Remove the depilatory cream thoroughly with wet gauze [4].
 - 3.3.1. Talent applying ophthalmic ointment to both eyes of the mouse.
 - 3.4.2. Talent attaching surface electrocardiogram electrodes to the limbs using electrode gel and securing them with tape **NOTE: This shot is moved here as per the author's request**
 - 3.3.2. Talent applying depilatory cream to the thoracic region.
 - 3.3.3. Talent removing the depilatory cream with wet gauze.
- 3.4. Rinse the skin gently with warm saline if necessary and dry the area [1]. ~~Attach surface electrocardiogram electrodes to the limbs using electrode gel at the contact sites and secure them with tape [2].~~
 - 3.4.1. Talent rinsing the skin with warm saline and drying the area.
 - 3.4.2. ~~Talent attaching surface electrocardiogram electrodes to the limbs using electrode gel and securing them with tape~~ **NOTE: 3.4.2 along with the corresponding VO is moved before 3.3.2**

4. Conventional Echocardiography Image Acquisition

- 4.1. Position the mouse in a horizontal, supine posture on the heated stage, with a slight leftward tilt if needed to optimize cardiac imaging [1]. Apply a generous amount of pre-warmed ultrasound coupling gel to the hairless left precordial region, avoiding air bubbles [2].
 - 4.1.1. Talent positioning the mouse in a horizontal, supine posture on the heated stage.
 - 4.1.2. Talent applying pre-warmed ultrasound coupling gel to the hairless left precordial region.
- 4.2. Place the transducer gently on the chest wall at the left parasternal position [1]. Adjust its orientation so that the index mark points toward the mouse's right shoulder [2] and fine-tune the angle and pressure to optimize image quality [3].
 - 4.2.1. Talent placing the transducer on the chest wall at the left parasternal position. NOTE: 4.2.1 and 4.2.2 are filmed in a single shot
 - 4.2.2. Talent orienting the transducer with the index mark directed toward the mouse's right shoulder.
 - 4.2.3. Talent adjusting the transducer angle and pressure while imaging. NOTE: 4.2.3 and 4.3.1 are filmed in a single shot
- 4.3. Then, switch to B-mode imaging [1], adjust the depth, focus zone, and frame rate to obtain a clear parasternal long-axis view of the left ventricle [2]. Confirm clear visualization of the endocardial border with no dropouts [3].
 - 4.3.1. B-mode is selected on the ultrasound imaging system.
 - 4.3.2. Depth, Focus Zone, and Frame Rate settings are being adjusted to optimize the parasternal long-axis view. NOTE: 4.3.2 and 4.3.3 are filmed in a single shot
 - 4.3.3. Endocardial border being clearly visualized in the parasternal long-axis view without image dropouts.
- 4.4. Once the heart rate stabilizes, acquire three separate parasternal long-axis B-mode cine loops, each containing at least 3 to 5 consecutive cardiac cycles [1]. Save each loop by pressing Cine Store [2] and store representative still frames using Frame Store [3].
 - 4.4.1. Parasternal long-axis B-mode cine loops are being acquired while cardiac cycles are recorded.
 - 4.4.2. Cine Store is being pressed to save a cine loop.
 - 4.4.3. Frame Store is selected to save representative still frames.
- 4.5. From the long-axis view, rotate the transducer 90 degrees clockwise to obtain a short-

axis view at the mid-papillary level [1]. Acquire three separate short-axis B-mode cine loops [2].

4.5.1. Talent rotating the transducer 90 degrees clockwise .

4.5.2. Short-axis B-mode cine loops are being acquired at the mid-papillary level.

4.6. Activate M-mode [1], position the cursor perpendicular to the interventricular septum and posterior wall [2], and record three separate M-mode tracings [3].

4.6.1. M-mode being activated on the ultrasound imaging system

4.6.2. M-mode cursor being positioned perpendicular to the interventricular septum and posterior wall.

4.6.3. Three separate M-mode tracings being recorded.

4.7. Use VevoLab 3.2.0 to measure interventricular septum thickness, left ventricular internal diameter [1], and left ventricular posterior wall thickness during diastole and systole from M-mode tracings, following the American Society of Echocardiography's leading-edge method [2].

4.7.1. SCREEN: 71451_screenshot_1.mp4: 00:20-00:35

4.7.2. SCREEN: 71451_screenshot_1.mp4: 00:36-00:54

4.7.3. SCREEN: 71451_screenshot_1.mp4:00:55-00:62

4.8. Calculate the left ventricular ejection fraction and fractional shortening using the Teichholz formula [1].

4.8.1. TEXT ON A PLAIN BACKGROUND

$$EF (\%) = [(LV \text{ Vold} - LV \text{ Vols}) / LV \text{ Vold}] \times 100, LV \text{ Vold} = ((7 / (2.4 + LVIDd)) \times LVIDd^3), LV \text{ Vol}s = ((7 / (2.4 + LVIDs)) \times LVIDs^3), FS (\%) = [(LVIDd - LVIDs) / LVIDd] \times 100.$$

5. Speckle-Tracking Strain Analysis

5.1. Perform speckle-tracking analysis using the dedicated analysis software [1]. Open a clear parasternal long-axis B-mode cine loop, then select Vevo Strain to enter strain analysis mode [2].

5.1.1. SCREEN: 71451_screenshot_2.mp4: 00:03-00:16

5.1.2. SCREEN: 71451_screenshot_2.mp4: 00:19-00:28.

5.2. Retain only complete cardiac cycles and choose Long Axis [1]. Manually trace the endocardial border at the end-diastolic frame [2].

5.2.1. SCREEN: 71451_screenshot_3.mp4: 00:00-00:12

5.2.2. SCREEN: 71451_screenshot_3.mp4: 00:13-00:16, 00:30-00:35

5.3. Adjust the automatically generated epicardial border as needed [1]. Then, start the

strain analysis and activate the toggle contour/vector/orbit line/B mode *(toggle contour-vector-orbit-line- B-mode)* function to visualize the motion trajectory [3].

5.3.1. SCREEN: 71451_screenshot_4.mp4: 00:02-00:06, 01:00-01:06

5.3.2. SCREEN: 71451_screenshot_4.mp4: 01:30-01:40.

5.4. Assess the tracking quality via the color-coded mesh [1]. Confirm that the mesh appears green, closely follows the endocardial and epicardial borders without jerking, and moves synchronously with myocardial contraction and relaxation [2].

5.4.1. SCREEN: 71451_screenshot_5.mp4: 00:00-00:05

5.4.2. SCREEN: 71451_screenshot_5.mp4: 00:06-00:11

5.5. Activate Time-to-peak analysis to divide the left ventricle into six segments, with each color representing an individual segment [1].

5.5.1. SCREEN: 71451_screenshot_6.mp4: 00:03-00:20.

5.6. Once satisfactory tracking is achieved, export the longitudinal and radial wall motion data for velocity, displacement, strain, and strain rate [1].

5.6.1. SCREEN: 71451_screenshot_7.mp4: 00:04-00:30

Results

6. Results

- 6.1. Echocardiographic assessment on postoperative day 7 revealed pronounced pathological cardiac remodeling in wild-type mice after myocardial infarction compared with sham-operated controls [1].
 - 6.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the images labelled 'WT Sham' and 'WT MI' in both the systolic and diastolic rows.*
- 6.2. Wild-type mice after myocardial infarction showed thinning of the interventricular septum [1] with significant left ventricular dilation as reflected by increased left ventricular internal diameter in diastole [2], increased left ventricular internal diameter in systole [3], and thinning of the posterior wall [4].
 - 6.2.1. LAB MEDIA: Figure 2B and C. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'IVSd(d) (mm)' and 'IVS(s) (mm)'.*
 - 6.2.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'LVID(d) (mm)'.*
 - 6.2.3. LAB MEDIA: Figure 2E. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'LVID(s) (mm)'.*
 - 6.2.4. LAB MEDIA: Figure 2F and G. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'LVPW(d) (mm)' and 'LVPW(s) (mm)'.*
- 6.3. These structural alterations were associated with a pronounced decline in global systolic function, as demonstrated by reduced ejection fraction [1] and reduced fractional shortening [2].
 - 6.3.1. LAB MEDIA: Figure 2H. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'EF (%)'.*
 - 6.3.2. LAB MEDIA: Figure 2I. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'FS (%)'.*
- 6.4. *Cracd*-deficient mice displayed significantly smaller left ventricular internal diameter in diastole as well as in systole compared with wild-type mice after myocardial infarction [1].
 - 6.4.1. LAB MEDIA: Figure 2D and E. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'LVID(d) (mm)' and 'LVID(s) (mm)'.*
- 6.5. *Cracd*-deficient mice exhibited improved systolic thickening of the posterior wall [1],

- maintained higher ejection fraction values [2], and higher fractional shortening values [3].
- 6.5.1. LAB MEDIA: Figure 2G. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'LVPW(s) (mm)'.*
 - 6.5.2. LAB MEDIA: Figure 2H. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'EF (%)'.*
 - 6.5.3. LAB MEDIA: Figure 2I. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'FS (%)'.*
- 6.6. Wild-type mice after myocardial infarction exhibited reduced radial velocity [1], reduced longitudinal velocity [2], reduced radial displacement [3], and reduced longitudinal displacement [4].
- 6.6.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Radial Velocity (m/s)'.*
 - 6.6.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Longitudinal Velocity (m/s)'.*
 - 6.6.3. LAB MEDIA: Figure 3D. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Radial Displacement (mm)'.*
 - 6.6.4. LAB MEDIA: Figure 3E. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Longitudinal Displacement (mm)'.*
- 6.7. The absolute values of radial strain [1], radial strain rate [2], longitudinal strain [3], and longitudinal strain rate were markedly decreased in wild-type mice after myocardial infarction [4].
- 6.7.1. LAB MEDIA: Figure 3F. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Radial Strain (%)'.*
 - 6.7.2. LAB MEDIA: Figure 3G. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Radial Strain rate (1/s)'.*
 - 6.7.3. LAB MEDIA: Figure 3H. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Longitudinal Strain (%)'.*
 - 6.7.4. LAB MEDIA: Figure 3I. *Video editor: Highlight the data points for 'WT Sham' and 'WT MI' on the graph labelled 'Longitudinal Strain rate (1/s)'.*
- 6.8. *Cracd*-deficient mice displayed better recovery of radial displacement [1] and longitudinal displacement compared with wild-type mice after myocardial infarction [2]. Furthermore, radial velocity [3], radial strain [4], and radial strain rate [5] were maintained at higher levels, while longitudinal strain [6] and longitudinal strain rate were significantly improved [7].
- 6.8.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the data points for 'WT MI' and*

'Cracd-def MI' on the graph labelled 'Radial Displacement (mm)'.

- 6.8.2. LAB MEDIA: Figure 3E. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'Longitudinal Displacement (mm)'.*
- 6.8.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'Radial Velocity (m/s)'.*
- 6.8.4. LAB MEDIA: Figure 3F. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'Radial Strain (%)'.*
- 6.8.5. LAB MEDIA: Figure 3G. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'Radial Strain rate (1/s)'.*
- 6.8.6. LAB MEDIA: Figure 3H. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'Longitudinal Strain (%)'.*
- 6.8.7. LAB MEDIA: Figure 3I. *Video editor: Highlight the data points for 'WT MI' and 'Cracd-def MI' on the graph labelled 'Longitudinal Strain rate (1/s)'.*

1. CRISPR/Cas9
Pronunciation link: <https://www.merriam-webster.com/medical/CRISPR>
IPA: /'krɪs.pə/ ; /kæz 'nɑm/
Phonetic Spelling: kris·per ; kaz·nine
2. Cracd
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: krak·dee
3. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>
IPA: /,aɪ.sou'flɒr.əm/
Phonetic Spelling: eye·soh·floor·ayn
4. Orotracheal
Pronunciation link: <https://www.merriam-webster.com/medical/orotracheal>
IPA: /,ɔ:r.ou'treɪ.ki.əl/
Phonetic Spelling: or·oh·tray·kee·uhl

5. Intravenous
Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>
IPA: /ˌɪn.trəˈviː.nəs/
Phonetic Spelling: in·truh·vee·nuhs
6. Ventilator
Pronunciation link: <https://www.merriam-webster.com/dictionary/ventilator>
IPA: /ˈven.təˌleɪ.tər/
Phonetic Spelling: ven·tuh·lay·ter
7. Ophthalmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
IPA: /ɑːfˈθæ.l.mɪk/
Phonetic Spelling: off·thal·mik
8. Corneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/corneal>
IPA: /ˈkɔr.ni.əl/
Phonetic Spelling: kor·nee·uhl
9. Depilatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/depilatory>
IPA: /dɪˈpɪl.əˌtɔːr.i/
Phonetic Spelling: dih·pil·uh·tor·ee
10. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/medical/povidone-iodine>
IPA: /ˌpɑː.vəˌdoʊn ˈaɪ.əˌdaɪn/
Phonetic Spelling: pah·vuh·dohn eye·uh·dyne
11. Pericardium
Pronunciation link: <https://www.merriam-webster.com/dictionary/pericardium>
IPA: /ˌper.əˈkɑːr.di.əm/
Phonetic Spelling: pehr·uh·kar·dee·uhm
12. Auricle
Pronunciation link: <https://www.merriam-webster.com/dictionary/auricle>
IPA: /ˈɔːr.ɪ.kəl/
Phonetic Spelling: or·ih·kuhl
13. Atraumatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/atraumatic>
IPA: /ˌeɪ.trəˈmæt.ɪk/
Phonetic Spelling: ay·truh·mat·ik
14. Myocardial
Pronunciation link: <https://www.merriam-webster.com/dictionary/myocardial>
IPA: /ˌmaɪ.oʊˈkɑːr.di.əl/
Phonetic Spelling: my·oh·kar·dee·uhl
15. Occlusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/occlusion>
IPA: /əˈkluː.ʒən/
Phonetic Spelling: uh·kloo·zhuhn

16. Echocardiography
Pronunciation link: <https://www.merriam-webster.com/dictionary/echocardiography>
IPA: /,ek.oʊ.kɑːr.diˈɑː.grə.fi/
Phonetic Spelling: ek·oh·kar·dee·og·ruh·fee
17. Parasternal
Pronunciation link: <https://www.merriam-webster.com/medical/parasternal>
IPA: /,per.əˈstɜː.nəl/
Phonetic Spelling: pehr·uh·stur·nuhl
18. Interventricular
Pronunciation link: <https://www.merriam-webster.com/dictionary/interventricular>
IPA: /,ɪn.təˈvenˈtrɪk.jə.lə/
Phonetic Spelling: in·ter·ven·trik·yuh·ler
19. Teichholz
Pronunciation link: <https://www.howtopronounce.com/teichholz>
IPA: /ˈtaɪk.hoʊlts/
Phonetic Spelling: tyke·holts
20. Epicardial
Pronunciation link: <https://www.merriam-webster.com/dictionary/epicardial>
IPA: /,ep.əˈkɑːr.di.əl/
Phonetic Spelling: ep·uh·kar·dee·uhl