We would like to thank the JoVE editorial team and the manuscript reviewers for their careful reading of our protocol. A number of issues were raised with the initial submission that we have corrected. In general, the editorial team and reviewers asked us to provide more detail on several components of the protocol, expand the introduction and discussion, and fix some grammatical errors. Please find our point-by-point response below.

**Editorial comments:**  
Changes to be made by the author(s) regarding the manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have reviewed the manuscript and corrected a number of proofreading mistakes.

2. Please expand your Introduction to include the following the advantages over alternative techniques with applicable references to previous studies.

The Introduction has been expanded to include the following:

Using this technique, individual or small groups of cardiac cells isolated from a donor embryo can be microinjected into a variety of regions of a host embryonic heart eliminating the need for extensive host preparation and the large tissue insults that arise using standard grafting techniques. The microinjection needles used for these implantation studies have an outer diameter of ~30-40 µm, which means the needle can be placed directly in the target tissue (i.e. can penetrate the embryonic myocardial wall) and cells can be focally delivered with minimal damage to the surrounding tissue. The protocol can be used to perform a variety of isotopic, heterotopic, isochoric, and heterochronic manipulations, providing a rapid, flexible, and low-cost approach to directly examine classical experimental embryological paradigms in the developing four-chambered heart.

3. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

We have corrected our scientific notation as requested.

4. Please include a space between all numerical values and their corresponding units: 15 mL, 37 °C, 60 s; etc.

We have corrected our spacing to meet the JoVE formatting requirements as requested.

5. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

The following ethics statement has been included:

All methods described adhere to animal care guidelines of The University of North Carolina at Chapel Hill.

6. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

7. 1.3: Please provide the composition of siliconizing agent. Please describe how to backload the solution into micropipette.

The siliconizing agent is commercially available and is listed in the Table of Materials.

8. 1.4: How to dry the glass capillaries and for how long?

The timing for drying the needles has been included.

9. 3.1, 4.1: Please specify the desired stage in this protocol.

The stage for manipulation has been included.

10. 3.2.4: Please provide the criteria for staging the embryo.

A reference to staging criteria (proposed by Hamilton and Hamburger) is now included.

11. 3.3: Is a syringe used to inject the mixture?

Details regarding the syringe are now included.

12. 4.2: How large is the petri dish?

Petri dish size is now included.

13. 5.3, 5.5: Please specify centrifugation parameters. Note that there is no step 3.7.

All centrifugation sets are as described in procedure 4.4. We have now indicated this in the text.

14. 5.4: Please provide the composition of the labeling dye solution.

The composition of the dye labeling solution is now provided in procedure 2.2

15. 6.2.1: How small is the incision?

Length of the incision is now provided in procedure 6.2.1

16. 6.3.2: Please describe how.

We are not sure what is being requested in the comment. This procedure is just visual inspection of the tissue.

17. Representative Results: Please describe all panels of Figure 1 in the text.

All panels are now described.

18. Discussion: Please discuss the significance with respect to existing methods and any future applications of the technique.

We have modified the discussion substantially to emphasize the points requested.

19. Figure 1: Please use the micro symbol µ instead of u and include a space between the number and the units of the scale bar (1 mm, etc.). In the figure legend, please define all abbreviations in the figure.

The figure and figure legend have been modified as requested.

20. A minimum of 10 references should be cited in the manuscript. For instance, please include applicable references to previous studies when describing advantages over alternative techniques.

We have added additional references.

21. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.

Table of materials has been reformatted.  
  
**Reviewers' comments:**  
  
Reviewer #1:  
  
Minor Concerns:  
  
There are a few inadequacies that might improve the manuscript, and I highlight those here along with some editorial suggestions:  
1) Silicone coating of the pipettes is novel, and likely helpful in preventing the frustration of needle plugs. However, the siliconizing reagents don't seem to be listed in the materials section. An image of a prepared needled might also be helpful.

The Siliconizing reagent is listed in the Table of Materials. We agree that an image of the needle would be helpful, and expect that the video recording of the procedure will be adequate for viewers.

2) A more detailed description of "backloading" of the injection needle would be helpful, since this can be a difficult procedure.

We have modified the text to provide more details regarding the materials needed to backload the needle and the process of doing so.

3) In line 41, the word "pression" is used. Because of the context, I initially thought this was a misspelling of "precision." Pression is an uncommon term, and perhaps the standard "pressure" could be used to avoid confusion by the reader.

This is, in fact, a grammatical error and we have corrected it. We did intend to use the term "precision".

4) In line 115 and 138, the phrase White Leghorn Horn Chicken eggs is used. Take out Horn as it is incorrect, unless this is a new breed. Alternatively, since the breed of chicken is likely not important, fertile chicken eggs would be an acceptable phrase.

The reviewer is correct, "horn" in this context was a typo. We have taken the reviewer's advice and simply used the term "fertile chicken eggs".

5) Line 118 describes a location on an egg shell, but it is unclear. Removing albumin from an egg usually occurs near the more pointed end, so as to avoid the air pocket on the more blunt end of the egg. Perhaps the authors could clarify. Perhaps an image would be helpful?

We have expanded the description of the text in this area of the protocol. We did not specify that we typically incubate the eggs in a horizontal orientation and puncture the blunt end for removing the albumen. This will be clear in the video recording.

6) Lines 181-182 describe the pressure for injection. Can the pulse length also be variable and modified? Is it important? Please clarify.

We typically use a series of 0.5 s pulses, however, the pressure is far more important for effective cell delivery. We have given a clearer description of this in the text.

7) Line 197 describes using PFA, but it is not in the materials list. Also, HBSS is not listed, or the ingredients to make it. Perhaps a better description of these reagents is warranted, since 4% PFA could be in water or in a salt solution like PBS or HBSS.

Hanks Balanced Salt Solution (HBSS) is included in the Table of Materials and we have added our procedure for making 4% PFA (procedure 2.3)

8) The discussion section seems to have written hastily. Please consider editing for sentence structure and flow. For example: line 221-222 - "drops in viability" could be changed to "reduced viability." Line 224 - "easiest" to "most". Line 226 - "too short a half-life for the application other…" is confusing. Line 229 - "…viable by time of injection." Needs modification.

We have rewritten the discussion, we hope that this draft is more consistent with the format requested by the JoVE editors.

Reviewer #2:  
  
Major Concerns:  
The authors should make explicit why is necessary to coat the glass capillaries with silicone.

We have added the following text to the manuscript:

NOTE: Coating the glass capillaries with silicone provides a chemically inert surface to the glass. If the capillaries are left untreated the cell suspension generated in later steps will adhere to the glass and plug the needle; therefore, coating is **NECESSARY** and **VITAL** to the success of the method.

It should also be clearly explained whether the tip of the glass needle needs to be in contact to the targeted tissue (if not, at which distance should it be?).

The needle tip should penetrate the target tissue. We have stated this in the revised procedure 6.3

Finally, is there any optimal cell concentration for this experiment? (will always work independently from the cell concentration in the injected suspension?).

We have poor injection quality at lower than 50,00 cells per ul. We have now stated this in the text (procedure 5.6).  
  
Minor Concerns:  
The protocol should provide alternatives. The use of indian ink to give some contrast during the injection (3.3) can be substituted by proper illumination with transmitted light (normally the light beam incidence angle should be of 90º with respect to the eyesight) or by using vital dyes like neutral red.

As the reviewer points out, there are many methods to increase contrast in the embryo. We felt that India ink would provide the best contrast for the video recording of the procedure (and that is why we included it). We have, however, referenced some alternative approaches as requested (procedure 3.3).