***In vivo* tissue engineering chamber for tissue construction with intrinsic vascularization (JoVE54099R2)**

We thank the editor and reviewers for his/her helpful and constructive comments. Please find our response below.

**Editorial comments:**

• Formatting  
-Please check that all portions of protocol steps are in the imperative tense (e.g., 1.1.2-“Adequate depth of anesthesia…toe-pinch.”; 1.3.7-“The proximal end…vein graft.”). In certain instances, such statements can be included as Notes.

This change has now been made.

-Please include catalog numbers in the Materials/Equipment list. In addition, please check that all items have been included in this list (e.g., cardiac troponin T antibody).

This change has now been made.  
  
•Additional detail is required:  
-1.2.11-Please elaborate on how to “close the wound in two layers.”

This has been clarified in Procedure.

-3.1-What are the typical time points used?

The typical harvesting time points are 4-6 weeks post-implantation. This has now been added to 3.1.

-3.3-Please elaborate on, or provide a superscript reference, for the test for vascular patency. In addition, how is the chamber removed? Do any specific cuts need to be made?

The test for patency has been described in step 3.3 of Procedure. The chamber is removed as explained after ligating the femoral vessels. No additional or specific cuts need to be made.

-3.5-Is fixation performed at room temperature or 4°C? In addition, provide a reference for microtome/cryostat sectioning.

Tissue fixation was performed at room temperature. This has been added to the manuscript (Please see page 7). Reference for microtome/cryostat sectioning has been added to the manuscript (Please see page 7).

-3.6-Provide references for immunohistochemistry.  
References have been added to the manuscript (Please see page 7).

•Branding should be removed:  
-Results-MBF Bioscience, CAST system (Olympus), Invitrogen

The branding has been removed.

-Figure 2 legend-Adipogel

The branding has been removed.

•JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

DOIs have been included for all references.

**Reviewer #2:**   
1. The authors have shown vascularity of a tissue constructs at 4 weeks post-implantation in figure 3 and transplanted cells in figure 4. However, it needs to clarify if there is any significant difference in the vascular number or even more transplanted tissue regenerated within this chamber model when compared with none (no chamber or no vascular pedicle). I suggest more statements or control data would be helpful as a supplementary for the representative results.

The chamber creates an ischemic environment that stimulates angiogenic sprouting from the implanted vessels. Cells are attracted to the chamber and tissue spontaneously grows until the space fills or encapsulation occurs. From our experience, no tissue grows if no vessel is implanted or the vascular pedicle thromboses. Equally if the vessel pedicle is simply implanted into normal tissue and not inside the protected space of the chamber, angiogenic sprouting ceases along the same timeline as a normal wound and no new tissue will accumulate around the pedicle.

We have added this to the Introduction (page 3) and Discussion (page 9).

2. The authors declared that a human-specific Ku-80 antibody was used to identify human IPS cells in Figure 4 (B). How to avoid the rejections in a rat model as it is not an immumocomprimised model? Authors should present the particular timepoint for harvesting and give the descriptions.  
Human iPS cells were implanted inside the tissue engineering chambers in immunocompromized rats and the tissues were harvested at 28 days post-implantation. This has been added to the manuscript (Please see page 8 and Figure 4 legend).  
  
**Reviewer #3:**   
1. Have the authors proven definitively that "angiogenic sprouting occurs from the arteriovenous vessels"? The authors should be clear about whether this is true and how it was determined.

We have previously conducted a time course experiment and showed that angiogenic sprouting first occurred from the femoral vein at 3-7 days followed by sprouting from the artery at day 10. Although blood vessel sprouting was not evident from the vein graft in the first 14 days, it occasionally occurred at slightly later time points. Capillary sprouts derived from the artery and vein were found to develop toward the vein graft and coursed along its abluminal surface and out into the chamber space.

[Lokmic Z, et al. An arteriovenous loop in a protected space generates a permanent, highly vascular, tissue-engineered construct. FASEB J.2007. **21:**511-22.]

This reference has now been added to the Introduction section (page 3, paragraph 4).

2. The authors should be clear that these procedures take a reasonable level of surgical skill, particularly the AV loop. I am not sure all basic researchers could pull it off without training even with this protocol.  
This has been clarified in the Discussion (Please see page 9).

**Reviewer #4:**   
1) While the vascularization aspect of this project is obvious, what is lacking is commentary on the subsequent steps needed to make this a clinically relevant platform. It is not clear to me whether the tissue would have to be harvested and subsequently re-transplanted at the injury/disease site, or whether the in-vivo vascularization would take place at the desired site and then chamber subsequently removed (or both depending on the case) In the case of the former, is harvesting possible without tissue morbidity?

Experimentally, the chambers have been typically sited in the groin because of the availability of large vessels for anastomosis in small animals. Subsequent transplantation to a more desirable site has been performed to confirm proof of principle (Tee R, Morrison WA, et al. Tissue Eng Part A. 2012. 18:1992-9) (Dolderer JH, et al. Plast Reconstr Surg. 2011. 127:2283-92). However, in the human clinical scenario, it would be preferable and much more feasible to create the chamber at the definitive site for reconstruction such as the breast, etc. We have done this in the first human trial of breast tissue engineering (Neopac) (presented at the International Breast Cancer Congress, Sydney 2012, and the manuscript is currently under reviewed by Annals of Surgery). We have added this to the Introduction (page 3).  
  
2) While I can imagine this technique leading to better vascularization and tissue integration, no controls were used to compare to current approaches.   
From our experience, no tissue grows if no vessel is implanted or the vascular pedicle thromboses. Equally if the vessel pedicle is simply implanted into normal tissue and not inside the protected space of the chamber, angiogenic sprouting ceases along the same timeline as a normal wound and no new tissue will accumulate around the pedicle.

We have added this to the Introduction (page 3) and Discussion (page 9).