W2.29  
Queens’ Medical Research Institute,

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Edinburgh,

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22nd April 2013.  
marie-claire.edmunds@ed.ac.uk

Dear Miss Jarzylo,

Re: Peer Review comments for: JoVE50473R1.

I have addressed each of these comments in turn (black) under the original comment from the reviewer.

Veterinary Reviewer:

\*0:59 - Indicates that animals are anesthetized but does not mention drug. Drugs are detailed in the PDF text.

Drug name now mentioned in new audio.

\*1:08?- Absolutely not acceptable: "Chlorhexidine applied and allowed to dry?" It appears in both the text and video that the skin is only prepped once. It is standard practice to prep the skin 2-3 times. The current information provided insinuates that skin is only prepped once.

I have modified the video to include images of the skin preparation. This is how the skin was prepared. It is based on strong clinical evidence and is in-keeping current evidence-based guidelines in clinical practice and our local Department of Veterinary Services guidelines.[1-3](#_ENREF_1) I have performed over 100 of these operations and have had no wound infections. I took this comment very seriously and performed an extensive literature search to find any evidence behind multiple chlorhexidne skin applications but was unable to find any evidence. I have subsequently amended the text to include the specific chlorhexidine-alcohol preparation that I used and have asked that future researchers clarify with their Veterinary Department what is standard practice in their unit and adopt that policy accordingly.

\*5:23 - Improvement required: Use of the silicone sheeting. Please have the author add a statement the purpose of the silicone sheeting.

This is explained in the text:

“The fasciocutaneous portions of the rat TRAM flap are thin enough to permit the flap to take as a full thickness skin graft. To prevent this and to ensure that this is a true model of IRI a thin, flexible silicon sheet is placed underneath the fasciocutaneous portions of the flap.[4](#_ENREF_4) This step has been adopted by other researchers undertaking rat TRAM models.[5-7](#_ENREF_5)”

I have also added a statement to that effect in the audio accompanying the video.

\*1.1 (TEXT) - Improvement required: "chlorhexidine in 70% alcohol". Please have author clarify this statement, is the chlorhexidine being mixed with alcohol or are both agents being used independently? Noticed there has been recent data to support the use of Chlorhexidine mixed in 70% alcohol.

I have changed this as requested.

\*P 15-32 (TEXT) - Improvement required: The Figure #s are missing. Please add figure #s to each figure.

I believe this is a problem with the PDF created for peer review. I uploaded all Figures with their names in the correct order but this may not appear in the PDF. This is perhaps something that the technical team at JOVE could rectify.

Reviewers' comments:

Reviewer #1:

Major Concerns:

N/A

Minor Concerns:

These are very small calibre vessels - what ackland clamp pressure was used where these low pressure 3v and 3A clamps - was there a distinction between arterial and venous clamps used. Very often in microsurgical procedures before division and anastomosis of vessels and following anastomosis vessel dilators such as papavarin or indeed calcium channel blockers like Verapamil are used- was this the case after clamp release in your model? Further given that the vessel intima can easily be damaged by the use of these clamps were any vessels look at histologically or under the operating microscope to ascertain vessel intima damage? Why was the ischaemia reperfusion injury time 30 minutes given that in most microsurgical procedures the duration of ischaemia is 60 to 120 minutes - to re establish microsurgical flow in under 45 minutes in free tissue transfer is unusual. Finally given that the rat has a very low volume of anterior abdominal fat - will this model reflect the role of IRI in free tissue transfer for breast reconstruction given that Free TRAM flaps or pedicled or indeed DIEAP flaps contain large fat volumes required for breast reconstruction? -this rat model looks at the anterior abdominal skin and as an end point.

I have amended the reagents list to give more details of the clamps. B-1, “V”-type clamps were used on both the artery and vein. These are the smallest and gentlest Acland clamps available and are suitable for vessels 0.4-1 mm in diameter, they exert a pressure of between 5-15 gm/mm2. We did not employ Verapamilor papavarine as we did not encounter prohibitive vasospasm but I thank the reviewer for the comment and have added it to the written protocol. After the clamps were removed re-establishment of arterial flow was confirmed by observing arterial flow over forceps. I was unable to get good video of this procedure otherwise I would have include it. The flaps were checked twice a day for any signs of venous congestion suggestive of venous thrombosis but none were found. I did assess a number of these vessels after the animals were culled at 48 for signs of intimal damage. It was difficult to tell exactly where the clamps had been placed as they left no marks but no evidence of intimal damage was evident in those that I studied. The ischaemic time was limited to 30 minutes due to stipulations by the UK Home Office authority that we had to work under. I have added to the text that this can be extended to cause more significant injury. The rat does have a low fat volume compared to many women undergoing TRAM flaps but not all free flaps have thick subcutaneous fat depending on the site and the habitus of the patient. This is a model primarily of free tissue transfer rather than specifically free tissue transfer for breast reconstruction. My aim is that it will be used to evaluate methods for improving outcomes in myocutaneous free tissue transfer but I have gone on to do a study to assess a pharmacological preconditioning strategy in this model (soon to be submitted to *Am. J. Pathol.*). I have changed the text accordingly to indicate the limitations of this TRAM specifically as a model of breast reconstruction.

Additional Comments to Authors:

The video and the explanation of the model is very clear and will serve as excellent model

Reviewer #2:

Major Concerns:

Major concerns - written part:

\* The authors are required to discuss "future applications" more in depth as this demonstrates the significance of the model.

This has been expanded.

\* The authors used LDI perfusion scanning to assess blood flow in the TRAM flap however do not refer to former publications where LDI scan has been used.

These references have been added.

\* In Figure 10 and 11, the authors provide representative results. The number of cases shown is low (n=3). A higher number and a statistical analysis would certainly strengthen the work.

I have changed these graphs (text and video) and text to reflect n =10.

Major concerns - video:

\* As this is supposed to be a video publication, we expect at least some differentiated photographs instead of only one picture of the experimental setup in the first minute.

Please find the video amended accordingly.

\* Anesthesia and skin preparation are well described in the written protocol however these steps are not shown in the video.

Please find the video amended accordingly.

\* The authors provide a schematic drawing of flap dimensions. They do not demonstrate the steps in the animal though fundamental for a video publication.

Please find video amended accordingly.

\* Magnification of the TRAM flap preparation should partially be reduced as the area of interest disappears out of sight.

Please accept my apologies but I am afraid this cannot be changed. I am no longer doing these surgeries and these are the best images that I have. If the JOVE video and manuscript review process, prior to peer review, had been shorter I could have taken more images. Equally, if the cost of their video assistance had been less I would have gladly used it.

\* Ideally, the authors in person should briefly discuss indications and limitations of the model at the end of the video.

We are contractually bound by the University of Edinburgh to not appear in person linking us with live animal work nor to film any identifiable buildings. I cannot, therefore, comply with this request but have added some audio to the video file to briefly outline this area. This is due to genuine fear of safety of researchers doing work on animals in the UK from anti-vivisection campaigners. The University has had two attempted bombings on these grounds in the recent past.

\* In summary non-microsurgical video sequences are essentially missing.

Please find updated images on the video.

Minor Concerns:

\* Figure 1 looks rather confusing and unprofessional. Legends and arrows might help.

I have added arrows to this images and updated the sequence of images to take in other comments.

\* The figures showing skin necrosis in the movie and the written part differ. Consistent graphs should be used.

Please accept my apology for this, I have updated these images.

\* The first section of "critical steps" should rather be part of the protocol text than the discussion as surgical instructions are described.

This text has been transferred to the end of the Protocol section as requested.

\* Explain "FTT". Free tissue transfer?

This has been added to the introduction.

\* Write "Isoflurane" instead of "Isoflourane".

Corrected.

Additional Comments to Authors:

N/A

Yours sincerely,

signature 130313_3.jpg

Miss Marie-Claire Edmunds BSc (Hons) MBChB (Hons) MRCSEd

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Bibliography

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