**Editorial comments:**

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**The manuscript has been revised by a professional editing company (Editage) and a certificate confirming this is included with our resubmission.**

2. Please revise the title to avoid puns.

**Title has been changed to “Novel *in vivo* tool for biomaterials validation: Example of intra-omental islet transplantation, h-Omental Matrix Islet filliNG (hOMING)”**

3. Please spell out each abbreviation the first time it is used.

**The revision complies with this comment.**

4. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

**The revision complies with this comment.**

5. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

**The revision complies with this comment.**

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:  
**The revision complies with this comment.**

1.1.3: How to carry out a C-peptide dosage/measurement?

**The measurement of C-peptide was done using an ELISA kit. We have included the following in the revised manuscript:**

**“**1.2.4. Include rats in the cohort when a measure of tail vein blood C peptide level is maintained under 200 pM one month after insulin pellet implantation. *Note: Checking c-peptide levels is mandatory to evaluate animals at baseline before transplantation and to confirm their diabetic state. Therefore, a low C-peptide regeneration always occurs during follow-up. The lowest C-peptidemia is indicative of the lowest regeneration.* ”

1.1.4: Please specify how to perform chronic insulin therapy.

**Chronic insulin therapy is done using insulin pellet implantation. Please see the explanation of the procedure in the subsection 1.2. (1.2.1 to 1.2.4).**

1.2.1: Please mention how animals are anesthetized and how proper anesthetization is confirmed.

**Anesthesia was accomplished using 3% isoflurane. The information has been added to the text as follows (lines 143 and 144): “**Anesthetize the rat using gas anesthesia (3% isoflurane in 500 mL/min O2) and place the rat in the prone position.”

1.2.3: Following this step, please discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.

**Following pellet implantation, no post-surgical treatment was administered, as this surgical intervention is comparable to a single subcutaneous injection. An explanation has been added to the revised text as follows (lines 153-155): “***Note: Recovery was extremely rapid as the intervention was only 2 min in length. No post-surgical pain management was necessary as the intervention was comparable to a single subcutaneous (SC) injection.”*

2.1.1: Please mention how to prepare alginate 1.5%, e.g., the volume to be prepared.

**Alginate was prepared from powder using sterile PBS. Four hundred microliters were needed per recipient. To clarify this information, the sentence was modified as follows (lines 172-175: “**Dissolve alginate powder in sterile PBS at a concentration of 1.5%. Sterilize the preparation by passage through a 0.22 µm filter. Prepare 400 µL per recipient. *Note: Any kind of hydrogel with a viscosity suitable for injection through a 21-G needle can be used.”*

2.1.2: Please spell out CMRL, IEQ, etc.

**The full terms for CMRL (Connaught Medical Research Laboratories) and IEQ (Islet Equivalent) have now been provided at the first use.**

2.2.4, 3.3: Please specify the surgical instruments used.

**Incision was made using a scalpel. The revised passages now read: “**Make an incision using a scalpel and remove the insulin pellet using forceps.” (line 191) and **“**Create a 1.5 cm laparotomy just under the sternum using a scalpel.” (line 252)

7. 2.2.2: Please break up into sub-steps.

**There are now three sub-steps. (2.2.2. to 2.2.4.).**

8. Please mention what happens to the rats at the end of the protocol.

**Rats were euthanized using pentobarbital overdose. This information has been included in the revision as follows (lines 285-286):** “3.8. Euthanize the rat 10 to 12 days after explantation using an overdose of pentobarbital (182.2 mg/kg).”

9. Please include histological analysis and describe eosin/hematoxylin staining of omental section in the protocol because such data are presented in the Representative Results.

**An additional section has been added (lines 279-282):**

**“4. Histological analysis: hematoxylin and eosin staining**

Fix the retrieved omenta using 4% paraformaldehyde (PFA) and embed in paraffin. Cut sections 4 µm in thickness and apply hematoxylin and eosin stain for morphological evaluation of the transplant.”

10. Please reference Figure 1 in the protocol.

**A reference to Figure 1 was added as follows in section 3.6 (lines 261-266): “**Continue excision along the part attached to the stomach and retrieve the omentum. *Note: At this location, gastroepiploic arteries (****Figure 1****) can cause a large amount of bleeding if they are accidentally cut. Artery incisions are inevitable to retrieve the graft, but bleeding can be managed using forceps and gauze. If an accidental cut happens, compress firmly with dry gauze and maintain the compression for at least 1 min. The bleeding should stop. If not, you can use clips or can use an electric bistoury to cauterize the vessels.”*

11. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

12. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.  
13. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

**Points 11 to 13: The revised text has been highlighted as you asked.**

14. Line 280: Should Figure 1 actually be Figure 2?

**The correction has been made.**

15. Figure 2: Please describe what different panels represent in the figure legend.

**Figure 2 has been changed as suggested to provide a more visual explanation of the method. Accordingly, the figure legend has been revised as follows:**

**"Figure 2. Description of hOMING technique and bead distribution through the omental tissue one day after implantation.**

(A) Illustration of the hOMING technique. After organ exposure (A, left), the islet-hydrogel mix (replaced here by blue-colored dextran beads for better visualization) was carefully injected in the tissue using an atraumatic needle (A, middle). Beads implanted in the tissue are visible (A, right). (B) Hematoxylin and eosin staining of omentum explanted 1 day after bead injection. Beads are found in the tissue with a uniform distribution. Scale bar=100 mm."

16. References: Please do not abbreviate journal titles.

**The references have been updated and the JoVE style in endnote corrected.**

17. Please remove the embedded Table of Materials from the manuscript.

**The table has been removed.**

**Reviewers' comments:**

**Reviewer #1:**

Manuscript Summary:

This protocol by Schaschkow and colleagues describes the procedure of transplanting islet/hydrogel mixture into the omentum. While this is not a new procedure, having it available through JoVE can be useful to other scientists. However, the protocol as currently written requires major revision to address the following shortcomings/concerns:

Major Concerns:

1- Information and representative results presented on transplanting beads during initial setup of this technique are not necessary and distract from the message.

**Figure 2 was changed to ensure clarity. Pictures of injection methods using beads are now provided. Colored beads were used as an easy visual representation of islet distribution in the omental tissue using hOMING. The revised figure legend reads as follows:**

**"Figure 2. Description of hOMING technique and bead distribution through the omental tissue one day after implantation.**

(A) Illustration of the hOMING technique. After organ exposure (A, left), the islet-hydrogel mix (replaced here by blue-colored dextran beads for better visualization) was carefully injected in the tissue using an atraumatic needle (A, middle). Beads implanted in the tissue are visible (A, right). (B) Hematoxylin and eosin staining of omentum explanted 1 day after bead injection. Beads are found in the tissue with a uniform distribution. Scale bar=100 mm."

2- More relevant information and results on the actual procedure with islets/hydrogel mixture are needed. For example, although the procedure will be highlighted in the video, images showing the exteriorized omentum can add much needed information to this protocol.

**A portion of the protocol was modified accordingly to ensure clarity of the procedure using the hydrogel/islet mix. Taking in account your first comment and this one, a picture showing the procedure of implantation have been added to the manuscript for clarification (Figure 2). The picture clearly depicts the exteriorized omentum and the schematics of the technique. The figure legend has been modified accordingly as described in the preceding point.**

3- Many statements/expressions throughout the protocol are vague, unsupported, or confusing and require further clarification. To name a few examples: what is the meaning of "power evaluation" in line 88; insulin "pumps" are suddenly mentioned in step 2.2.2, why; line 374 "technique showed benefits in term of reproducibility", how?; line 368 "viscosity has to be tested in an empty device before use", what device?; line 378 "transplantation into the liver, which can cause risks in terms of hepatic function", like what?

**We have incorporated your suggestions as shown by the blue text on lines 86-87 and 89.**

**Line 86-87: “**Therefore, currently there are no tools to quickly, safely, and efficiently evaluate a beneficial effect of a hydrogel.”

**Line 191: “**Make an incision using a scalpel and remove the insulin pellet using forceps**”**

**Concerning reproducibility, the rats that underwent hOMING and all displayed graft function. This was not the case if we considered, as two example, intra-hepatic transplant or sub-cutaneous transplant.**

**Line 412, which concerns viscosity testing, was modified. The empty syringe refers to a cell-free hydrogel injectability test conducted using the syringe. As this original description was not clear, we revised the sentence by clearly mentioning that viscosity had to be tested before use. The revised sentence reads: “**Liquid hydrogel will leak out of omental tissue and highly viscous hydrogel will not be injectable. Hydrogel viscosity and injectability has to be tested before use, but it appears essential to also test the material directly by evaluation of islet or other cells survival in vitro.”

**Concerning the risks in terms of hepatic function, which include local steatosis and/or inflammation, can affect hepatic enzyme function. This is clarified in the revised sentence and a supporting reference is provided. The sentence reads (lines 421-422): “**Also, hOMING allows the transplanting of islets into a “non-vital” organ, unlike transplantation into the liver, which can cause risks in terms of hepatic function12” The reference: Leitao, C. B. *et al.* Liver fat accumulation after islet transplantation and graft survival. *Cell Transplant* **23**, 1221-1227, doi:10.3727/096368913X668663 (2014).

4- Figure numbers are mixed up in the text. Figure legends must be updated to better explain the presented results. Information on animal numbers (n=?) and statistical analyses is missing.

**Figures numbers have been corrected and the legends revised. Animal numbers and statistical analyses are mentioned in the figure legends and in the main text.**

**“**we performed isogenic studies using Lewis rats (n=8)”

**“5. Statistical analysis**

Statistical significance was determined using Statistica software (Statsoft, Maisons-Alfort, France) and repeated measures analysis of variance (ANOVA) with Tukey’s honest significance difference test as a post hoc test and is represented as follows: \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.”

5- Any reference in the protocol to omental "folds" should be avoided and instead use omental "layers" as this is very confusing since it implies folding the omentum, which is an established procedure in islet transplantation in the omentum. Clearly, in the current protocol the omentum is not folded, correct?

**You are correct. That is exactly the point that we are showing in this paper; the absence of folding with this technique is facilitated by an injection between adipocytes. As suggest, "fold" has been replaced by "layers".**

**“**cell-hydrogel mixture between the omental layers, in close proximity to blood vessels,”

**“**which consists of the injection of the graft inside the tissue, in between the omental layers,”

Minor Concerns:

Certain items in the actual protocol need revision and additional clarification:  
1- In step 1.2.4, is checking c-peptide levels absolutely necessary or just checking glycemia to confirm diabetes (hyperglycemia) is sufficient?

**Checking the C-peptide levels is mandatory to obtain baseline values prior to transplantation and to evaluate the real impact of the graft on diabetes. Metabolic follow-up of C-peptide on sham animals that had not received grafts revealed that low C-peptide regeneration always occurred. To be sure of the lowest level of regeneration, animals with C-petidemia exceeding 200 pM were not included in the study. Furthermore, a pump was used to maintain the animals during the month preceding transplantation. Also, a hypoglycemic effect can subsist somewhat after pellet retrieval. Thus, measurement of C-peptide provides reassurance of graft function when compared to pre-transplant levels. This information is clarified in the revision of 1.2.4 as follows (lines 156-159):**

**“**1.2.4. Include rats in the cohort when a measure of tail vein blood C-peptide level is maintained under 200 pM one month after insulin pellet implantation. *Note: Checking C-peptide levels is mandatory to evaluate animal baseline values before transplantation and to confirm their diabetic state. Low C-peptide regeneration always occurs during follow-up. The lowest C-peptidemia is indicative of the lowest regeneration.* ”

2- Define IEQ at first use.

**IEQ stands for islet equivalent. This full term has now been supplied.**

3- In Step 2.1.2, it is not clear whether the islets are pelleted and resuspended directly in the 150ul hydrogel or in media before adding the hydrogel.

**Islet aliquots are washed and the supernatant is discarded. The islets are then dispersed 150 µL of hydrogel are added on islet. The sentence has been revised to make this information clear (lines 178-181):** “In a laminar flow hood, prepare islet aliquots of 7660-islet equivalent (IEQ)/kg body weight. Wash islets with CMRL (Connaught Medical Research Laboratories) medium free of FBS. The islets are recovered by centrifugation and the supernatant is discarded. The islets are dispersed in 150 µL of hydrogel carrier and the mixture is placed on ice.”

4- In step 2.2.2, insulin pellet removal should be included in an earlier step to confirm diabetes/hyperglycemia prior to the transplant procedure.

**We chose to conserve the insulin pellet until transplantation for several reasons. Firstly, the maintenance of a proper glycemic control considerably reduced the oxidative stress induced by diabetes in the recipient organs, which can be deleterious for islets graft (see Schaschkow et al, Journal of Diabetes Research 2016) and allows the continuation of intensive insulin therapy and recipient preparation observed in the clinic. Secondly, we wanted to limit the number of anesthesia procedures that rats had to endure. Thirdly, as glycemia is not a relevant parameter if rats are under insulin therapy using pellets, we monitored C-peptide dosage at the beginning of the study to select animals (<200 pM) after STZ injection and examined a control the day before transplantation to ensure that beta cells had not regenerated and the rats remained diabetic.**

5- "pumps" are suddenly mentioned in step 2.2.2, did u mean insulin pellets?

**The correction you suggest has been made. Thank you.**

6- Step 2.2.3 should include the instruction to place the rat in the supine position prior to surgical site preparation.

**The sentence containing this information have been moved to its original place at the beginning of the next paragraph. “**2.2.5. Place the rat in the supine position. Shave and sterilize (povidone iodine) the peritoneal area. Let the iodine stand for 3 min.”

7- Steps 2.2.5 and 3.4 should include clear instruction to exteriorize (pull out) the omentum prior to spreading on the gauze.

**We acknowledge your comments and have revised the text as follows (lines 210-214):** “Use forceps to carefully catch the omentum, pull it gently out of the peritoneal cavity, and spread it on the gauze. The omental tissue extends from the spleen to the duodenum and attaches at it mid-point to the stomach. The normal omentum of diabetic rats receiving insulin therapy is approximately 2 cm² when spread on the gauze. ”

8- Step 2.2.7 should explain HOW islets/hydrogel mixture is "properly dispersed into the tissue". Is this with the needle, by finger massaging, how?

**The proper dispersion was ensured by using the needle movement during injection. As this sentence was not clear, we have revised it as follows (in 2.2.9.): “**Inject the islet preparation slowly and carefully move the needle backward to inject in several places (as lines). Ensure that the hydrogel stops exiting the needle to avoid loss of islets dispersed in the hydrogel.**”**

9- Step 2.2.9 must explain more clearly whether injecting "all the hydrogel contained in the device" should be done before or after the multiple injections at the different entry points. It should also address whether any leaks of the islet/hydrogel mixture can be expected upon multiple injections, and how to address this if it occurs.

**The injection of the islets dispersed in the hydrogel had to be done several times. Each entry point allowed the delivery of a small amount of the islets. The needle was inserted as far as possible and the injection is started as the needle was moved backward to form a line. When approaching the end of the tissue, the needle movement was paused for a short time to ensure that no further extrusion of hydrogel from the needle occurred. Then the needle was retrieved and inserted in another part of the omentum and the manipulation was repeated until the syringe was empty.**

**Concerning leakage, the viscosity of the hydrogel is important. Since the hydrogel is very viscous, leaks will not occur if the necessary precaution of waiting time is observed. Clarification has been provided in 2.2.9 and 2.2.10 as follows:**

**“**2.2.9. Inject the islet preparation slowly and carefully move the needle backward to inject the islets in several places (as lines). Prior to withdrawing the needle, ensure that the hydrogel has stopped exiting the needle to avoid loss of the dispersed islets.

2.2.10. Repeat this manipulation as much needed to inject the entire contents of the syringe using different entry points to distribute the islets throughout the omental tissue. *Note: In rats, four to five injections are generally needed.***”**

10- Step 2.2.11 should emphasize sterility of saline.

**We apologize for the omission. The, adjective "sterile" has been added.**

11- In step 2.2.15, is giving insulin in the first few days absolutely required during these initial few days after transplant? would this potentially limit the ability to verify graft function based on glycemia? This should be clarified.

**Insulin treatment is mandatory in islet transplantation. Glucotoxicity created by hyperglycemia can extensively impair graft function and destroy the graft before it becomes functional. This is the reason why insulin therapy is realized in the measurement of glycemia. As soon as glycemia exceeded 2 g/L, rats received an insulin injection. An injection was not given at levels less than 2 g/L. Glycemia measurements were done daily at 9 am, with the rats being on an inverted cycle (with night being from 10 am to 10 pm; night is their active phase). When glycemia was measured in the morning, the rats were still in their passive phase and were not feeding extensively, which created a sort of "fasting" glycaemia. As soon as glycemia was measured, insulin was injected if needed. The insulin formulation used was slow insulin (Lantus). Preliminary experiments have tested the duration of action on rats. The result showed that 24 hours later insulin was not detectable. Thus, even if rats were injected daily with insulin, the glycemia measurements would not be impaired by the injection even 24 hours later. The timing involved needed to be precise. The insulin therapy we chose was deliberate, and did not impair glycemia measurements.**

**Discussion about the insulin therapy have been added in the revised manuscript as follows (lines 409-423):**

"The conservation of proper glycemia management (before transplant using pellets and thereafter using long-acting insulin) is mandatory. Here, we chose to conserve insulin pellet until the day of transplantation for several reasons. First, the maintenance of a proper glycemic control considerably reduces oxidative stress induced by diabetes in the recipient organs, which can be deleterious for islet graft11 and allows continuation of intensive insulin therapy and recipient preparation observed in the clinic12. Second, we wanted to limit the maximum number of anesthesia procedures for the rats. Concerning the implementation of insulin therapy during the metabolic follow-up using long-acting insulin, the scheme used avoided massive complications due to glucotoxicity (which can destroy grafts ) and to conserve a “real” glycemia value.

To monitor graft efficiency, glycemia is not a relevant parameter in the first few days if rats are receiving insulin therapy using pellets. This is why checking the C-peptide level at several times prior to transplant is mandatory. These times include at the beginning of the study, to select animals after STZ injection, and then just preceding transplantation, to ensure that rats remain diabetic and that regeneration is minimal. "

12- Avoid vague terminology and use more precise terms in your description of the procedures. For example, line 280 "nice distribution", does this mean even or uniform distribution?

**As suggested, “nice” has been replaced by “uniform”. “**

13- Authors are strongly encouraged to consult with a native English-speaker to address grammatical and contextual errors throughout the protocol that have the potential to misguide the reader.

**The manuscript has been revised by a professional editing company (Editage) and a certificate confirming this is included with our resubmission.**

**Reviewer #2:**

Although this represents an interesting line of research, the data provided and analysis do not appear to justify a full report. There are so many omissions of details that it would not be possible for a reader to reproduce the studies as they are written. On top of that there are numerous technical issues with the way experiments were performed. Other major deficiencies are multiple examples of grammar problems and unclear statements throughout the paper. The authors should do a careful review of the entire manuscript looking for and correcting errors, or find someone who can do it for them.

I only point out several specific issues below although there are numerous other issues.  
  
# The authors removed insulin pellet and transplant islets during one surgical procedure. This means that blood glucose level just prior to transplant would not be high. However, in Figure 3A, the authors show extremely high blood glucose level prior to transplantation. This strange data presentation will detract from the validity of the entire experiments.

**The value representing high glycemia is the value after the induction of diabetes and before placement of insulin pellets. We chose to include this value on the graphic to better visualize glycemic changes observed during the study that were consistent with the scheduled experiment. After the induction of diabetes, the high glycemia was regulated until the time of transplant (time 0) using pellets. When the pellets were retrieved, the maintenance of glycemia at a normal level attested to the graft function. When graft was removed and pre-pellet implantation was resumed, the diabetic state also resumed. As these details can be misunderstood, we changed the description of the decrease of glycemia by maintenance of normoglycaemia after insulin pellet retrieval as follows (lines 315-316): “**After transplantation and insulin pellet retrieval, glycemia maintenance and normalization was observed just three days post-hOMING transplantation, and was maintained until graft retrieval (p<0.05 compared to pre-transplant levels).”

# The authors treated rats with insulin pellet for one month before transplant. It is totally not clear why the authors took this approach. It is generally possible to conduct transplant about 1 week after diabetes induction using STZ.

**Insulin pellet were used for several reasons, as mentioned in the text (point 1.1.4.). First, we previously demonstrated that chronic insulin therapy of rats before transplantation improves the diabetic state (weight loss, organ failure, oxidative stress in the recipient site). Second, to perform a high quality transplant, the omental fat needs to be sufficiently extensive to accept the graft. We previously performed studies using rats examined just after the induction of diabetes and under chronic insulin therapy, and demonstrated that one month appeared to be the best treatment time to obtain sufficient omental fat pad for graft and to avoid diabetic complications.**

# The authors state that omental graft explantation is optional. I disagree. This is the necessary procedure to confirm normoglycemia was due to graft functioning.

**We agree and have removed the word “optional” from our protocol.**

# In Figures 3A and 3B, the authors failed to explain what a grey shaded area indicates and what the asterisks indicate. Furthermore, there is no information as to how many recipients were used.

**The gray shaded areas represent the maximal and the minimal values recorded for each time point. Eight animals were used as recipients. Information as added in to the figure legend as follows: “Figure 3. Two-month metabolic follow-up of rats receiving hOMING and graft assessment.**

(A) Glycemia measurement and (B) C-peptide assessment after hOMING using alginate as an islet carrier (Tx: Transplantation and insulin pellet retrieval; Explantation: Explantation of the omentum). Grafts are functional, as shown by the maintenance of normoglycemia after insulin pellet retrieval and increase in C-peptidemia after islet implantation. Grey shaded areas represent the minimal and maximal recorded values at each time point. (C) Hematoxylin and eosin staining of an omental section after islet transplantation using the hOMING method. Islets are well-integrated into the tissue two months after implantation without any surrounding fibrotic tissue. Vessels have grown around and inside islets, as shown by the arrows, and thus completely restore islet function. Morphology of the islets also seems well-preserved. Scale bars are 50 µm. (n=8) (\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001 determined using repeated measures analysis of variance (ANOVA) with Tukey’s honest significance difference test as a post hoc test). **”**

**Reviewer #3:**

Manuscript Summary:

A novel method of intra-omental islet transplantation using hydrogel in rat model. The authors succeeded to engraft the islets and improve the endocrinal function of the islet transplanted rats.

Major Concerns:

Please show how to transplant islets using hydrogel (needs to show by figure and movie). The methodology of transplantation is the most important of this paper.

**A figure was changed to present in greater detail how to perform the transplant using hOMING (Figure 2). The video supports the text.**

**"Figure 2. Description of hOMING technique and bead distribution through the omental tissue one day after implantation.**

(A) Illustration of the hOMING technique. After organ exposure (A, left), the islet-hydrogel mix (replaced here by blue-colored dextran beads for better visualization) was carefully injected in the tissue using an atraumatic needle (A, middle). Beads implanted in the tissue are visible (A, right). (B) Hematoxylin and eosin staining of omentum explanted 1 day after bead injection. Beads are found in the tissue with a uniform distribution. Scale bar=100 mm."

**Reviewer #4:**

Manuscript Summary:

Overall a JOVE manuscript with a video would be useful to the scientific community

Major Concerns:

1.) The introduction section needs a more in depth review of literature. Omental islet transplantations are not new and some have been done including some using hydrogels. A few are listed below.

a.) Berman et al Long-term survival of nonhuman primate islets implanted in an omental pouch on a biodegradable scaffold. Am J Transplant. 2009 Jan; 9(1): 91-104.  
b.) Hefty et al Omental roll-up: a technique for islet engraftment in a large animal model. J Surg Res. 2010; 161(1):134-138. ( plasma coagulation gel)  
c.) Kim HI, Yu JE, Park CG, Kim SJ. Comparison of four pancreatic islet implantation sites. J Korean Med Sci. 2010;25(2):203-210 (no hydrogel)  
d.) Kin T, Korbutt GS, Rajotte RV. Survival and metabolic function of syngeneic rat islet grafts transplanted in the omental pouch. Am J Transplant. 2003;3(3):281-285.

**The use of omentum as a recipient site is not new. However, the islet implantation technique is. This technique is inspired from a reconstructive surgical procedure (see Schaschkow et al., Cell Transplant. 2018). The entire cited examples used “hard” hydrogels that are not suitable for an injection as the one presented in our manuscript.**

2.) Line 52-53 - "Syringes loaded with ....cells" Cells should be "Islets"

**The change has been made.**

3.) Line 86-87 - "...sub-cutaneous is not optimal" needs more explanation as SC may not be "optimal" it is a less invasive procedure which does have some clinical advantage. See Gebe et al Local, Controlled Release In Vivo of Vascular Endothelial Growth Factor Within a Subcutaneous Scaffolded Islet Implant Reduces Early Islet Necrosis and Improves Performance of the Graft.

**SC implantation might be considered an alternative in terms of invasiveness. However, venous drainage will never be physiologic as for sub-cutaneous insulin injection. For proper islet function, is has been demonstrated that a portal drainage is more efficient. For clarity, the following passage for the venous aspect of the claim has been added (lines xx-xx): “**However, when grafted cells are meant to regulate systemic factors by metabolic action, this sub-cutaneous localization is not optimal, especially in terms of venous drainage7.”

3.) Line 93 states "...first step was to identify an ideal transplantation site...", as no comparison was made to other transplantation sites the authors cannot claim to have identified an ideal site.

**The following revision was made: “**The first step was to identify an acceptor transplantation site,”

4.) Line 95-96 ""...studying cells bearing metabolic activity..." as all cell have metabolic activity perhaps add "high" metabolic activity such as islet cells.

**The following revision was made: “**Omentum offers a large space for implantation, it is highly plastic, and the dense vascularization combined with the intraperitoneal situation is interesting for studying cells bearing high metabolic activity8.”

5.) While islet isolation is not the focus here the authors should give reference to where this could be obtained. How did they obtain their islets?

**Islets were obtained as previously described using healthy Lewis rats, and involved collagenase digestion of the pancreas infused through the bile duct and a gradient separation. A reference has been added for how islets were obtained. The revised passages reads (line 164): "** Islet-matrix mixture preparation. Islet isolation was done as previously described10." Reference: Schaschkow, A. et al. Impact of an autologous oxygenating matrix culture system on rat islet transplantation outcome. Biomaterials 52, 180-188, doi:10.1016/j.biomaterials.2015.02.031 (2015).

6.) As you are using alginate as the demonstrated hydrogel, how is this prepared? Is it sterile filtered.

**Alginate was reconstituted from sterile powder using sterile PBS. It was filtered through a sterile a 0.22 µm before use with cells. The revised passage is as follows (lines 166-169): "** Prepare viscous islet carrier in laminar flow hood. Dissolve alginate powder in sterile PBS at a concentration of 1.5%. Sterilize the preparation by passage through a 0.22 µm filter. Prepare 400 µL per recipient. *Note: Any kind of hydrogel with a viscosity suitable for injection through a 21-G needle can be used.*"

7.) What is the concentration of the citrate buffer for STZ, is this solution filtered (sterilized)?

**The citrate buffer solution is 0.1 M. It was sterilized by filtration using a 0.22 µm filter.**

8.) Figure 1 and 3 need to be of higher resolution.

**The necessary improvements in resolution have been made.**

9.) In the Material / Equipment - list the alginate used and source

**Alginate was purchased from Novamatrix. The information has been included in the revised text.**   
10.) a 4th column is need in the Mat / Eq. table to explain what Ethilon and Metacam are as these words are not used in the main text (suture and meloxicam).

**Ethilon is a 4-0 suture. Metacam is the commercial name of Meloxicam, an non-steroidal anti-inflammatory drug. This information has been added to the revised table.**

11.) Oxygen as a material is listed in the Mat / Eq. table but is not used in the main text. Perhaps add "(for isoflurane use)"

**The details of isoflurane use have been added in the text and in the materials table.**

11.) Line 229 - readers might not know what "slow insulin" is, please explain and also list the long acting (slow) insulin in the Material / Equipment table.

**Slow insulin denotes long-acting insulin. The clarification has been made in the text and in the materials list.**

12.) Line 218 - is the suture the same for muscle wall and cutaneous layers?

**No, the sutures were different, as was mentioned in the text. The muscle was closed using a continuous suture and cutaneous layers were closed using a single point-by-point suture. This is now described in point 2.2.12.**

Minor Concerns:

1.) what is the "h" in hOMING acronym

**The h is added here to indicate the word “home”, as the use of an hydrogel nesting and the omentum as a proper recipient site can create a new “home” for islet. Please see our previous publication in cell transplantation for more detailed explanation (Schaschkow et al, 2018, Cell Transplantation).**

2.) Need to define "IEQ" (islet equivalents?)

**IEQ stands for islet equivalent and has been added to the text.**