Journal of Visualized Experiments

Intra-Omental Islet Transplantation using h-Omental Matrix Islet filliNG (hOMING)

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58898R2
Full Title:	Intra-Omental Islet Transplantation using h-Omental Matrix Islet filliNG (hOMING)
Keywords:	diabetes; omentum; islet transplantation; hydrogel; Innovative technique
Corresponding Author:	Elisa Maillard Universite de Strasbourg Strasbourg, FRANCE
Corresponding Author's Institution:	Universite de Strasbourg
Corresponding Author E-Mail:	e.pedracini@ceed-diabete.org
Order of Authors:	Anaïs Schaschkow
	Carole Mura
	Michel Pinget
	Karim Bouzakri
	Elisa Maillard
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Open Access (US$4,200)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	CEED, Boulevard René Leriche 67200 STRASBOURG, FRANCE

CENTRE EUROPÉEN D'ÉTUDE DU DIABÈTE

Boulevard René Leriche - 67200 Strasbourg - Tél. : 03 90 20 12 12 - Fax : 03 90 20 12 19
association@ceed-diabete.org - www.ceed-diabete.org

Unité Mixte de Recherche de l'Université de Strasbourg « Diabète et Thérapeutique »

Dear Editor,

I, along with my coauthors, would like to resubmit the attached manuscript entitled **"Novel in vivo tool for biomaterials validation. Example of intra-omental islet transplantation, hOMING (h-Omental Matrix Islet filliNG)",** manuscript ID JoVE58898

We appreciate the thorough reviews provided by the reviewers. We have revised the manuscript accordingly (revisions in the text are highlighted in blue). In addition, the manuscript has been carefully checked and edited by a professional service (Editage). Our point-to-point responses are attached in the file "Responses to Reviewers."

We hope that the revised manuscript is now suitable to be sent out for review.

I look forward to your reply.

Sincerely,

Elisa Maillard
UMR DIATHEC, EA 7294, Centre Européen d'Etude du Diabète
Boulevard René Leriche
67200 Strasbourg, France

CENTRE EUROPÉEN D'ÉTUDE DU DIABÈTE

Boulevard René Leriche - 67200 Strasbourg - Tél. : 03 90 20 12 12 - Fax : 03 90 20 12 19
association@ceed-diabete.org - www.ceed-diabete.org

Unité Mixte de Recherche de l'Université de Strasbourg « Diabète et Thérapeutique »

CERTIFICATE OF ENGLISH EDITING

This document certifies that the paper listed below has been edited to ensure that the language is clear and free of errors. The edit was performed by professional editors at Editage, a division of Cactus Communications.The intent of the author's message was not altered in any way during the editing process. The quality of the edit has been guaranteed, with the assumption that our suggested changes have been accepted and have not been further altered without the knowledge of our editors.

TITLE OF THE PAPER

Novel in vivo tool for biomaterials validation: Example of intra-omental islet transplantation, h-Omental Matrix Islet filliNG (hOMING)

AUTHORS

Anaïs Schaschkow, Carole Mura, Karim Bouzakri, Michel Pinget, Elisa Maillard

JOB CODE

ELMAI_1_2

editage by CACTUS

Signature

Vikas Narang,
Vice President, Author Services, Editage

Date of Issue
September 24, 2018

Editage, a brand of Cactus Communications, offers professional English language editing and publication support services to authors engaged in over 500 areas of research. Through its community of experienced editors, which includes doctors, engineers, published scientists, and researchers with peer review experience, Editage has successfully helped authors get published in internationally reputed journals. Authors who work with Editage are guaranteed excellent language quality and timely delivery.

DNV·GL ISO/IEC 27001

CACTUS

Contact Editage

Worldwide	Japan	Korea	China	Brazil	Taiwan
request@editage.com	submissions@editage.com	submit-korea@editage.com	fabiao@editage.cn	contato@editage.com	submitjobs@editage.com
+1 877-334-8243	+81 03-6868-3348	1544-9241	400-005-8055	0800-892-20-97	02 2657 0308
www.editage.com	www.editage.jp	www.editage.co.kr	www.editage.cn	www.editage.com.br	www.editage.com.tw

TITLE:

Intra-Omental Islet Transplantation using h-Omental Matrix Islet filliNG (hOMING)

AUTHORS & AFFILIATION :

Anaïs Schaschkow[1], Carole Mura[1], Karim Bouzakri[1], Michel Pinget[1], Elisa Maillard[1]

[1]Université de Strasbourg, Centre Européen d'Etude du Diabète, Strasbourg, France

Corresponding Author:

Elisa Maillard (e.pedracini@ceed-diabete.org)

Email Addresses of Co-authors:

Anaïs Schaschkow (a.schaschkow@gmail.com)

Carole Mura (c.mura@ceed-diabete.org)

Michel Pinget (m.pinget@ceed-diabete.org)

KEYWORDS:

Cell transplantation, Hydrogel, Innovative technique, Omentum, Diabetes, Islet transplantation, hOMING

SUMMARY:

Here, we present a protocol for *in vivo* validation of hydrogel-based cell therapy, illustrated by the example of islet transplantation. h-Omental Matrix Islet filliNG (hOMING) implantation allows implantation of a cell-hydrogel mixture between the omental layers, near to blood vessels, to maximize engraftment in a proper metabolic environment.

ABSTRACT:

Regenerative medicine based on cell therapy represents a new hope for curing disease. Current obstacles include proper *in vivo* validation of the efficiency of the therapy. For transfer to the recipient body, cells often need to be combined with biomaterials, especially hydrogels. However, validation of the efficacy of such a graft requires the right environment, the right hydrogel, and the right recipient site. The omentum might be such a site. Based on the example of islet transplantation, we developed the hOMING (h-Omental Matrix Islet filliNG) technique, which consists of the injection of the graft inside the tissue, in between the omental layers, to improve islet implantation and survival. To achieve this, islets have to be embedded in a hydrogel with a viscosity that enables its injection using an atraumatic needle. Syringes are loaded with a combination of hydrogel and islets. Several injections are performed inside the omental tissue at different entry points, and the deposition of the islet/hydrogel mixture is made along a line. We tested the feasibility of this innovative approach using dextran beads. The beads were well spread throughout the omental tissue, in close proximity to blood vessels. To test the efficacy of the graft, we transplanted islets into diabetic rats and perform a metabolic follow-up over two months. The transplanted islets exhibited a high rate of re-vascularization around and inside islets, and reversed diabetes. The hOMING technique could be applicable for other types of hydrogel or cell therapy, for cells with high metabolic activity.

INTRODUCTION:

Cell therapy is a hot topic, as it aims to cure diseases based on the regenerative medicine. Biological material-assisted cell therapies have been increasingly studied in recent years, especially because cell implantation often requires a carrier for the transfer of the cells from the culture dish to the recipient. Biomaterial scaffolds are potentially valuable cell carriers that fulfill several roles[1]. A competent carrier should protect cells from mechanical stresses and provide favorable growth conditions, such as essential growth factors, metabolic waste excretion, exchange of nutrient substances, and oxygen[2].

Among the different types of biomaterials used in cell therapy, hydrogels have many advantages. They are biocompatible, biodegradable, easy to handle, and facilitate oxygen diffusion[3]. Furthermore, current technology allows the use of hydrogels to help cells survive and engraft with, for example, supplementation with growth factors or extra-cellular matrix proteins[4].

Hydrogel carriers containing stem cells can be injected as treatments, *e.g.*, bone regeneration[5] and nervous system disease[6]. The implantation of metabolically active cells is needed. While *in vitro* validation of the approach is possible, tools and techniques for *in vivo* validation remain to be refined.

Cell and hydrogel transplantation can easily be carried out by subcutaneous injection when biocompatibility tests are conducted. However, when grafted cells are meant to regulate systemic factors by their metabolic action, this subcutaneous localization is not optimal, essentially in terms of venous drainage[7]. Therefore, there are no current tools to quickly, safely, and efficiently evaluate the beneficial effects of a hydrogel. Based on the example of islet transplantation, which requires that hormones be released into the bloodstream from the graft in response to blood glucose levels, we developed a new method for cell/hydrogel implantation *in vivo*.

The first step was to identify an acceptor transplantation site, which can accept a hydrogel with cells. The omentum offers a large space for implantation, is highly plastic, and its dense vascularization combined with the intraperitoneal setting is interesting for studying cells having high metabolic activity[8]. We next needed to establish a surgical technique allowing the transfer of the cells and the hydrogel into the omentum. Inspired by the lipofilling used in plastic surgery[9], we developed the h-Omental Matrix Islet filliNG (hOMING) approach. Islets embedded in hydrogel are injected inside the omental tissue. The technique also aimed to provide maximum engraftment by utilizing multiple depositions of the cell and hydrogel mixture into the omental tissue, where the large number of blood vessels also improves graft oxygenation.

In the present study, we describe a simple and innovative technique for islet implantation between omental sheets, inside the fat tissue closest to the blood vessels. This consists of micro-invasive surgery, which could be completed under laparoscopy, with the injection of islets contained in a hydrogel into the fat tissue. This technique is easily applicable to all hydrogel and cell combinations that need to be tested in a in a metabolically functional environment.

PROTOCOL:

All animal experiments were performed according to the National Institutes of Health guidelines, with the authorization number: AL/60/67/02/13.

1. Recipient Preparation

1.1. Chemically induce diabetes in recipient rats.

1.1.1. Inject 75 mg/kg of streptozotocin (STZ, in sterile 0.1 M citrate buffer, pH 4) intraperitoneally to rats[10].

NOTE: For the transplantation studies, 6-week old Lewis strain rats, weighing 150-190 g were used.

1.1.2. Check the diabetes status by daily blood glucose measurements during the first four days. Inject long-acting insulin 6 U/day subcutaneously when rats exhibit glycemia over 2 g/L to prevent diabetes complications and weight loss.

1.1.3. Include rats in the cohort when two measures of tail vein blood glucose are > 4-5 g/L for 2 consecutive days, and C-peptide level is > 200 pM. Measure glycemia using a glucometer and C-peptidemia by an enzyme-linked immunosorbent assay (ELISA).

1.1.4. Implant insulin pellets under the skin (see 1.2).

NOTE: Chronic insulin therapy allows better glycemia regulation and avoids diabetic complications (which exacerbate oxidative stress at the transplantation site)[11]. Furthermore, the therapy conserves the diabetic state along with a normal growth curve (without usual weight loss observed in a diabetic animal). This can result in a bigger omental fat pad, which is ideal for carrying out transplantation.

1.2. Implantation of insulin pellets

1.2.1. Anesthetize the rat using gas anesthesia (3% isoflurane in 500 mL/min O_2) and place the rat in the prone position.

1.2.2. Verify anesthesia status by checking the absence of reflex (paw pinching). Clean the neck using povidone iodine, and shave the area using a razor blade. Apply povidone iodine again and let it stand for 3 min.

1.2.3. Place 1.5 insulin pellets (3 Units (U)/200 g rat) in a 1:5 diluted povidone iodine solution to sterilize the pellets. Pierce the neck skin using a 16 G trocar and insert the pellet using the furnished guide and stylet. Retrieve the guide and stylet and stitch a single point. Use povidone iodine to clean the stitch.

NOTE: No post-surgical pain management was necessary as the intervention was comparable to a single subcutaneous (SC) injection.

1.2.4. Let the rat recover from anesthesia and ensure that rats have access to food to avoid hypoglycemia.

1.2.5. Measure the efficiency of pellet by measuring glycaemia decrease after implantation.

1.2.6. Check the pellet efficiency by monitoring glycemia level every week for 1 month.

1.2.7. Include rats in the cohort when a measure of tail vein blood C peptide level is maintained under 200 pM 1 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. Low C-peptide regeneration always occurs during follow-up. The lowest C-peptidemia is indicative of the lowest regeneration.

2. hOMING: Intra-omental Matrix Islet Filling

2.1. Islet-matrix mixture preparation.

2.1.1. Prepare the viscous islet carrier in a 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.

2.1.2 Isolate islet from healthy Lewis rats (200-250 g) as previously described[12].

2.1.3. Count islet number in islet equivalents (IEQ) (one IEQ is considered equivalent to a pancreatic islet with a diameter of 150 µm)[13].

2.1.4. In a laminar flow hood, prepare aliquots of 7660-islet equivalent (IEQ) in a 1.5 mL tube.

2.1.5. Wash islets aliquots with 500 µL of CMRL (Connaught Medical Research Laboratories) medium free of fetal bovine serum.

2.1.6. Pellet islets by centrifugation (2 min at 500 x g and 4 °C). Discard the supernatant.

2.1.7. Add 150 µL of alginate hydrogel carrier over the islets, mix carefully by pipetting up and down and place the mix on ice.

2.1.8. Prepare an atraumatic 21 G needle and a 1 mL syringe without dead volume by loading 150 µL of empty alginate into the syringe.

2.1.9. Fill the syringe with the mixture of islets and alginate (150 µL, total volume 300 µL). Keep the syringe on ice.

2.2. Surgical procedure

2.2.1. Sterilize surgical instruments using cold sterilization (2% Steranios for 20 min).

2.2.2. Anesthetize the rat using isoflurane anesthesia and place the rat in the prone position.

2.2.3. Shave the neck area using a razor blade and sterilize the area with povidone iodine. Let the iodine stand for 3 min.

2.2.4. Make an incision using a scalpel and remove the 1.5 insulin pellets using forceps. Close the skin using one or two single stitch points. Do not remove the rat from anesthesia.

NOTE: After 1 month, the pellet can be friable as some fibrotic tissue can wrap as pellets; use scissors to properly dissect it.

2.2.5. Place the rat in the supine position. Shave and sterilize (with povidone iodine) the peritoneal area. Let the iodine stand for 3 min.

2.2.6. Create a 1.5 cm laparotomy just under the sternum using a scalpel. Place wet-sterile gauze around the incised area.

2.2.7. Identify the omentum that is the fat pad localized next to stomach. Use forceps to carefully catch the omentum, pull it gently out of the peritoneal cavity, and spread it on the gauze.

NOTE: The omental tissue extends from the spleen to the duodenum and attaches at its midpoint to the stomach. The normal omentum of diabetic rats receiving insulin therapy is approximately 2 cm^2 when spread on the gauze.

2.2.8. Hydrate the omental tissue well using 2 mL of pre-warmed 37 °C sterile saline. Use small curved forceps to manipulate the tissue and penetrate the omental edge with the needle between the omental layers. Insert the needle entirely.

2.2.9. Start the injection of 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 the loss of the dispersed islets.

2.2.10. Repeat this manipulation as 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.

2.2.11. Check that islets are not clustered in the syringe at the end of the injections.

NOTE: If some islets are still visible, it is possible to withdraw the needle from the syringe, fill the syringe directly with 100 µL of empty hydrogel, and reconnect the needle. A second round of injection can be done to flush out the remaining islets.

2.2.12. Use sterile saline again to hydrate the omental tissue and the wall of the laparotomy. Use forceps to carefully replace omentum in the abdominal cavity.

2.2.13. Inject 2 mL of pre-warmed sterile saline into the abdominal cavity to rehydrate the rat.

2.2.14. Close the muscle wall using a continuous thread suture. Then stitch the cutaneous layer with single stitch point (point-by-point).

2.2.15. Inject meloxicam (1.5 mg/kg) subcutaneously as an analgesic for 5 days once a day.

2.2.16. Place the rat in a cage on a heating pad until recovery from anesthesia. Repeat the procedure for all the recipient rats.

2.2.17. Measure the blood glucose after transplantation every day. If glycemia is > 2 g/L, inject 6 U of long-acting insulin subcutaneously once a day.

2.2.18. Assess graft function by glycemia and c-peptidemia monitoring over 1 or 2 months.

NOTE: In cases of successful transplantation, glycemia should stabilize within 2-5 days after transplantation, and rats can be taken off insulin.

3. Omental Graft Explantation

NOTE: This procedure will permit the confirmation of good graft function. After retrieval of a functional graft, rats should return to a diabetic state. This step is performed after 1 or 2 months of metabolic follow-up.

3.1. Anesthetize the rat with gas anesthesia and place it in the supine position.

3.2. Shave the peritoneal area and sterilize it using povidone iodine for 3 min.

3.3. Create a 1.5 cm laparotomy just under sternum using a scalpel. Place wet-sterile gauze all around the incised area.

3.4. Identify the omentum, which is next to the stomach. Use forceps to carefully spread it on the gauze.

3.5. Use scissors to excise the omentum. Start from the part that adheres onto the pancreas tail (next to the spleen). If bleeding occurs, use dry sterile gauze to stop it.

3.6. 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, use clips or use an electric bistoury to cauterize the vessels.

[Place Figure 1 here]

3.7. Check if any bleeding persists. If not, inject 2 mL of pre-warmed saline, close the rat, and process as previously described.

NOTE: Explanted animals return to a diabetic state. Insulin injection (6 U/SC/day) is then mandatory to assure well-being of animals.

3.8. Euthanize the rat 10 to 12 days after explantation using an overdose of pentobarbital (182.2 mg/kg).

4. Histological Analysis: Hematoxylin and Eosin Staining

4.1. Fix the retrieved omenta using 4% paraformaldehyde (PFA) and embed in paraffin.

4.2. Cut sections 4 µm in thickness and apply hematoxylin and eosin stain for morphological evaluation of the transplant.

5. Statistical Analysis

5.1. Determine statistical significance using statistical analysis software and repeated measures analysis of variance (ANOVA) with Tukey's honest significance difference test as a post hoc test. Represent *p* values as: **p* <0.05; ** *p* <0.01; *** *p* <0.001.

REPRESENTATIVE RESULTS:

The hOMING method permits the avoidance of intravascular implantation and the confinement of islets in an organ. A maximum time of 8-10 min is required for the entire islet implantation procedure, including anesthesia, which a timeline comparable to classical liver transplantation.

To study the way islets are distributed inside the omental tissue, dextran beads were transplanted using the hOMING method (**Figure 2**). One day after implantation, rats were sacrificed, and omental tissues were retrieved for histological analysis. Hematoxylin and eosin staining revealed a uniform distribution of the beads throughout the tissue (**Figure 2,** bottom right). Very often, beads were close to blood vessels and were well-implanted in the fat tissue. Immediately after implantation, an inflammatory reaction occurs around the beads, resulting in tissue rearrangement to nest the islets in the tissue.

[Place Figure 2 here]

To validate this technique, we performed isogenic studies using Lewis rats (n=8). Diabetic rats receiving islet transplants (7660 islet equivalent, IEQ) per kg rat body weight using hOMING were monitored for glycemia and C-peptidemia for two months. Glycemia was controlled by implantation of insulin pellet (as attesting to the first drop in glycemia observed in the **Figure 3A**). Graft function was reflected by glycemia of approximately 2 g/L and C-peptidemia >500 pM. Before transplantation, rats were diabetic (glycaemia >5 g/L and C-peptidemia <200 pM). After transplantation and insulin pellet retrieval, glycemia maintenance and normalization was observed just 3 days post-hOMING transplantation and was maintained until graft retrieval (p<0.05 compared to pre-transplant levels). After omental explantation, glycemia rose again to the pre-transplant level, attesting to the functionality of islets that were transplanted by hOMING (**Figure 3A**). The C-peptidemia pattern was exactly the opposite, with low to undetectable levels before the graft, followed by an increase and maintenance at this increased level throughout the course of the study (p <0.05), and, after omental explantation, a decrease to pre-transplant levels (**Figure 3B**). Analysis of the explanted omentum by histology revealed highly re-vascularized islets, most likely as a result of their proximity to blood vessels (**Figure 3C**).

[Place Figure 3 here]

FIGURE AND TABLE LEGENDS:

Figure 1. Omental artery distribution. For omentum graft explantation, the critical area composed of gastroepiploic arteries is represented in blue. During resection of this part of the omental tissue, attention must be to be paid to the right gastroepiploic artery section. Compression, ligature, or cauterization can be used to limit bleeding.

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.

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 is shown by the maintenance of normoglycemia after insulin pellet retrieval and increase in C-peptidemia after islet implantation. Gray 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).

DISCUSSION:

Some critical steps can be highlighted in this protocol. First, the person performing the surgery and manipulating the tissues must be delicate with the fat tissue, as it is fragile. Crushing or damaging the omentum needs to be avoided. Omentum, as a defensive tissue, is enriched in macrophages and other leukocytes. These immune cells can be activated by excessive manipulation and could negatively affect the graft. Secondly, graft (islet-hydrogel mixture) loading is also critical. The person performing the procedure must avoid dead volumes and must load all of the hydrogel-islet mixture into the injection device. Immediately after this step, another critical point is the injection itself. Injection must be performed slowly, with care, and delicately. The syringe must be flushed by the empty hydrogel loaded initially to retrieve any remaining islets. Thirdly, suturing must be done carefully and in two steps. The muscular plan should be sutured first, taking care not to suture the omentum with the muscle, and the skin should be sutured separately. Regarding the explantation procedure for the omental graft, special attention should be taken at the moment when the arteries close to the stomach (gastroepiploic arteries) are incised. It is important to pay attention to any hemorrhages that occur and stop them before suturing the animal, as any persistent bleeding leads to animal death within several days.

Troubleshooting may be necessary regarding the medium for carrying islets; the choice of hydrogel is up to the experimenter, as long as the hydrogel is injectable. The use of different materials can result in variable graft function (with or without supplementation, for example). The method described here has proven its efficiency for inert co-transplanting material with an islet ratio of 7660 IEQ/kg.

The present method may be limited by the omental size and hydrogel properties. To use a diabetic rodent model, insulin therapy is mandatory prior to islet implantation to provide sufficient grafting area. Diabetic rodents lose a lot of weight and fat mass due to STZ-induced diabetes. Considering the small weight of the animals enrolled in this study, grafting in an even smaller area is not possible.

Conservation of proper glycemia management (before transplant using pellets and thereafter using long-acting insulin) is mandatory. Here, we chose to conserve the 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 graft[11] and allows continuation of intensive insulin therapy and recipient preparation observed in the clinic[12]. 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 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.

Hydrogel properties are also important. 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 another cells survival *in vitro*. Here, as islets are very sensitive to hypoxia and the use of a highly viscous carrier can affect oxygen diffusion.

In terms of the significance of the method described here with respect to existing methods, this intra-tissue transplantation technique has benefits in term of reproducibility. It is also atraumatic for the tissue and, because of its extra-vascular location, avoids bleeding and thrombosis risks. Also, when considering islet transplantation, instant blood-mediated inflammatory reaction is avoided[14]. Furthermore, hOMING allows transplanting islets into a non-vital organ, unlike transplantation into the liver, which poses risks in terms of hepatic function[15].

To produce optimal performance, a hydrogel has to be adapted to the cells it will carry. Use of growth factors or specific proteins can have a positive effect on transplanted cells[16,17].

To conclude, this technique can be used for *in vivo* validation of a hydrogel on different cell types, especially when an active metabolic environment is needed, as for islets. In addition, this surgical technique is applicable to multiple applications and could be, in the future, rapidly transferable to humans, as it can be easily performed using laparoscopy.

ACKNOWLEDGMENTS:
This work was funded by Région Alsace, BioArtMAtrix–Pôle Alsace Biovalley-CQDM; 53/14/C1. The authors are grateful to the team of Pr. Bruant-Rodier from the Hôpitaux Universitaires de Strasbourg for helping develop this innovative technique.

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:

1 Bakhshandeh, B. *et al.* Tissue engineering; strategies, tissues, and biomaterials.

Biotechnology & genetic engineering reviews **33**, 144-172, doi:10.1080/02648725.2018.1430464 (2017).

2 Lutolf, M. P., Gilbert, P. M. & Blau, H. M. Designing materials to direct stem-cell fate. *Nature* **462**, 433-441, doi:10.1038/nature08602 (2009).

3 Slaughter, B. V., Khurshid, S. S., Fisher, O. Z., Khademhosseini, A. & Peppas, N. A. Hydrogels in regenerative medicine. **21**, 3307-3329, doi:10.1002/adma.200802106 (2009).

4 Rice, J. J. *et al.* Engineering the regenerative microenvironment with biomaterials. *Advanced healthcare materials* **2**, 57-71, doi:10.1002/adhm.201200197 (2013).

5 Bai, X. *et al.* Bioactive hydrogels for bone regeneration. *Bioactive Materials* **3**, 401-417, doi:https://doi.org/10.1016/j.bioactmat.2018.05.006 (2018).

6 Allbright, K. O. *et al.* Delivery of adipose-derived stem cells in poloxamer hydrogel improves peripheral nerve regeneration. *Muscle Nerve*, doi:10.1002/mus.26094 (2018).

7 Van Der Windt, D. J., Echeverri, G. J., Ijzermans, J. N. M. & Cooper, D. K. C. The Choice of Anatomical Site for Islet Transplantation. *Cell transplantation* **17**, 1005-1014, doi:10.3727/096368908786991515 (2008).

8 Zweifach, B. W. & Lipowsky, H. H. Quantitative studies of microcirculatory structure and function. III. Microvascular hemodynamics of cat mesentery and rabbit omentum. *Circ Res* **41**, 380-390 (1977).

9 Bruant-Rodier, C., Dissaux, C., Baratte, A., Francois Fiquet, C. & Bodin, F. [The breast of the adolescent girl]. *Ann Chir Plast Esthet* **61**, 629-639, doi:10.1016/j.anplas.2016.05.006 (2016).

10 Schaschkow, A. *et al.* Extra-Hepatic Islet Transplantation: Validation of the h-Omental Matrix Islet filliNG (hOMING) Technique on a Rodent Model Using an Alginate Carrier. *Cell transplantation* **27**, 1289-1293, doi:10.1177/0963689718784873 (2018).

11 Schaschkow, A. *et al.* Impact of the Type of Continuous Insulin Administration on Metabolism in a Diabetic Rat Model. *Journal of diabetes research* **2016**, 8310516, doi:10.1155/2016/8310516 (2016).

12 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).

13 Kissler, H. J. *et al.* Validation of methodologies for quantifying isolated human islets: an Islet Cell Resources study. *Clinical transplantation* **24**, 236-242, doi:10.1111/j.1399-0012.2009.01052.x (2010).

14 Delaune, V., Berney, T., Lacotte, S. & Toso, C. Intraportal islet transplantation: the impact of the liver microenvironment. *Transplant international : official journal of the European Society for Organ Transplantation* **30**, 227-238, doi:10.1111/tri.12919 (2017).

15 Leitao, C. B. *et al.* Liver fat accumulation after islet transplantation and graft survival. *Cell transplantation* **23**, 1221-1227, doi:10.3727/096368913X668663 (2014).

16 Narang, A. S. & Mahato, R. I. Biological and biomaterial approaches for improved islet transplantation. *Pharmacological reviews* **58**, 194-243, doi:10.1124/pr.58.2.6 (2006).

17 Alvarado-Velez, M., Pai, S. B. & Bellamkonda, R. V. Hydrogels as carriers for stem cell transplantation. *IEEE Trans Biomed Eng* **61**, 1474-1481, doi:10.1109/tbme.2014.2305753 (2014).

Figure 1

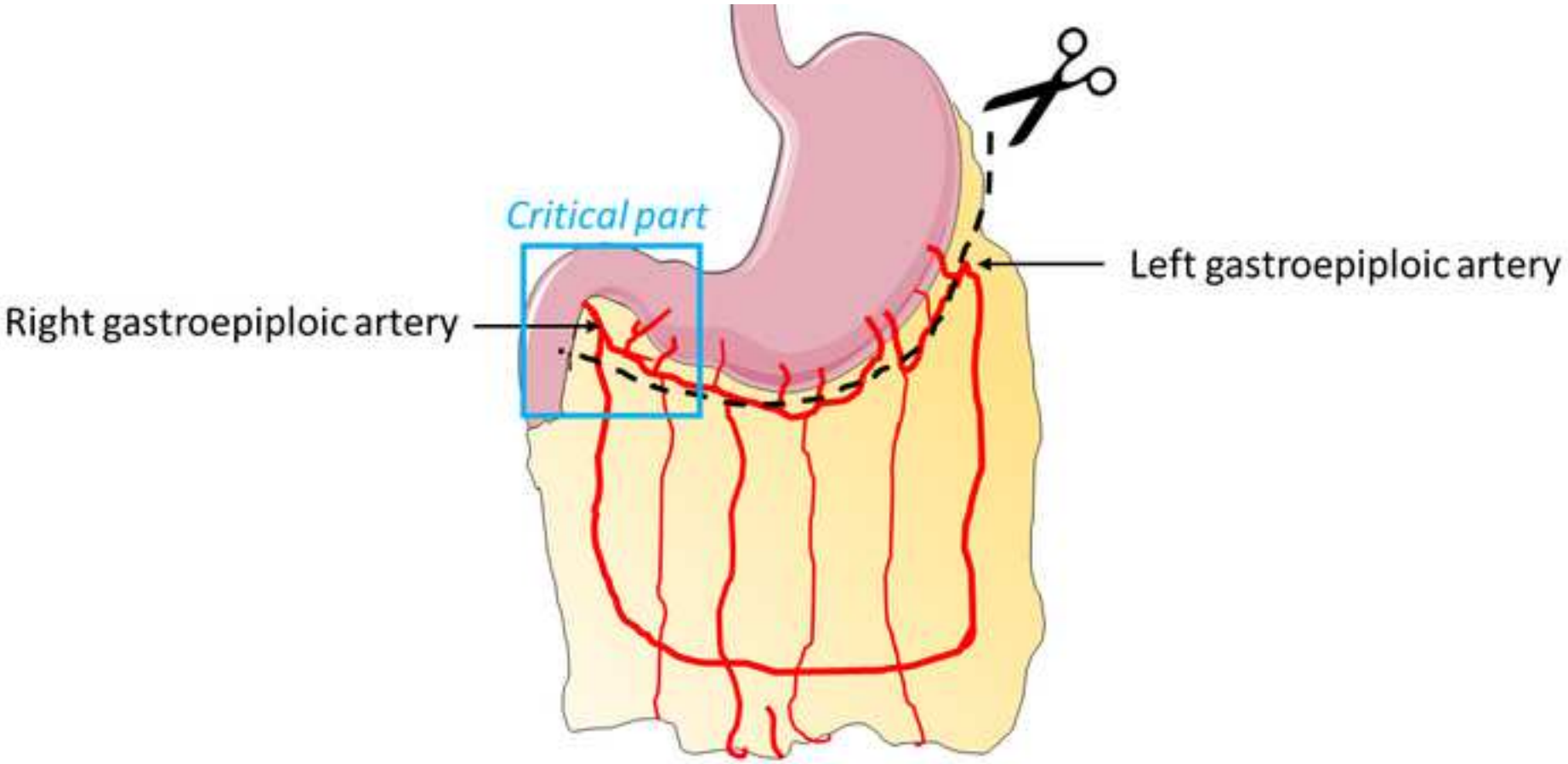


Figure 2

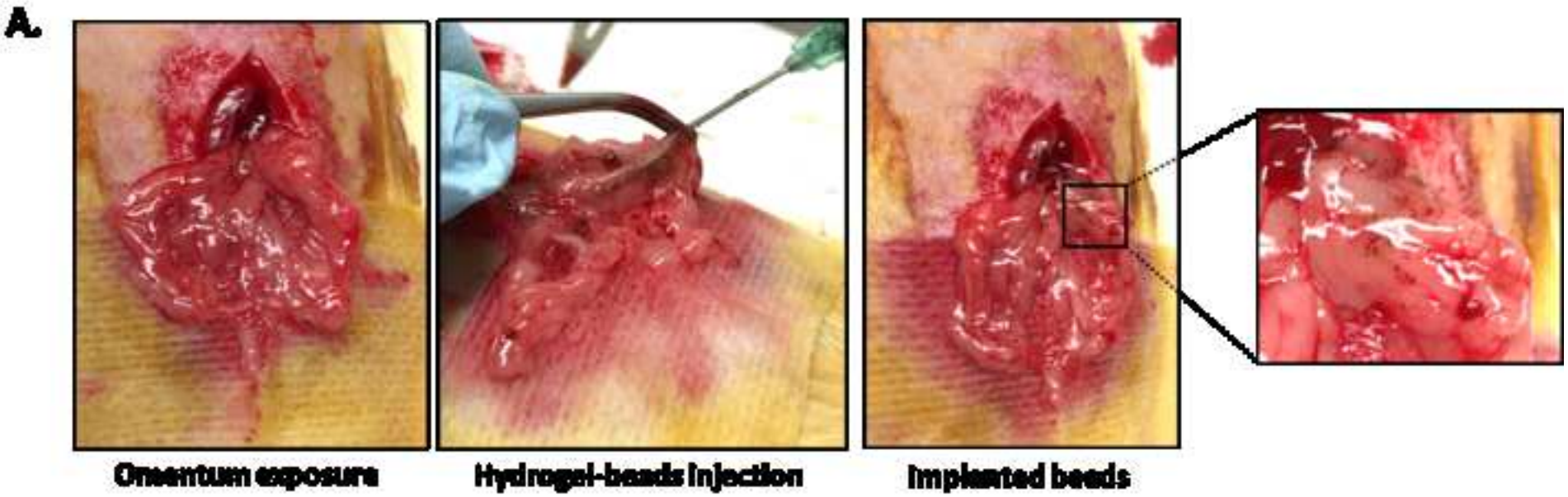
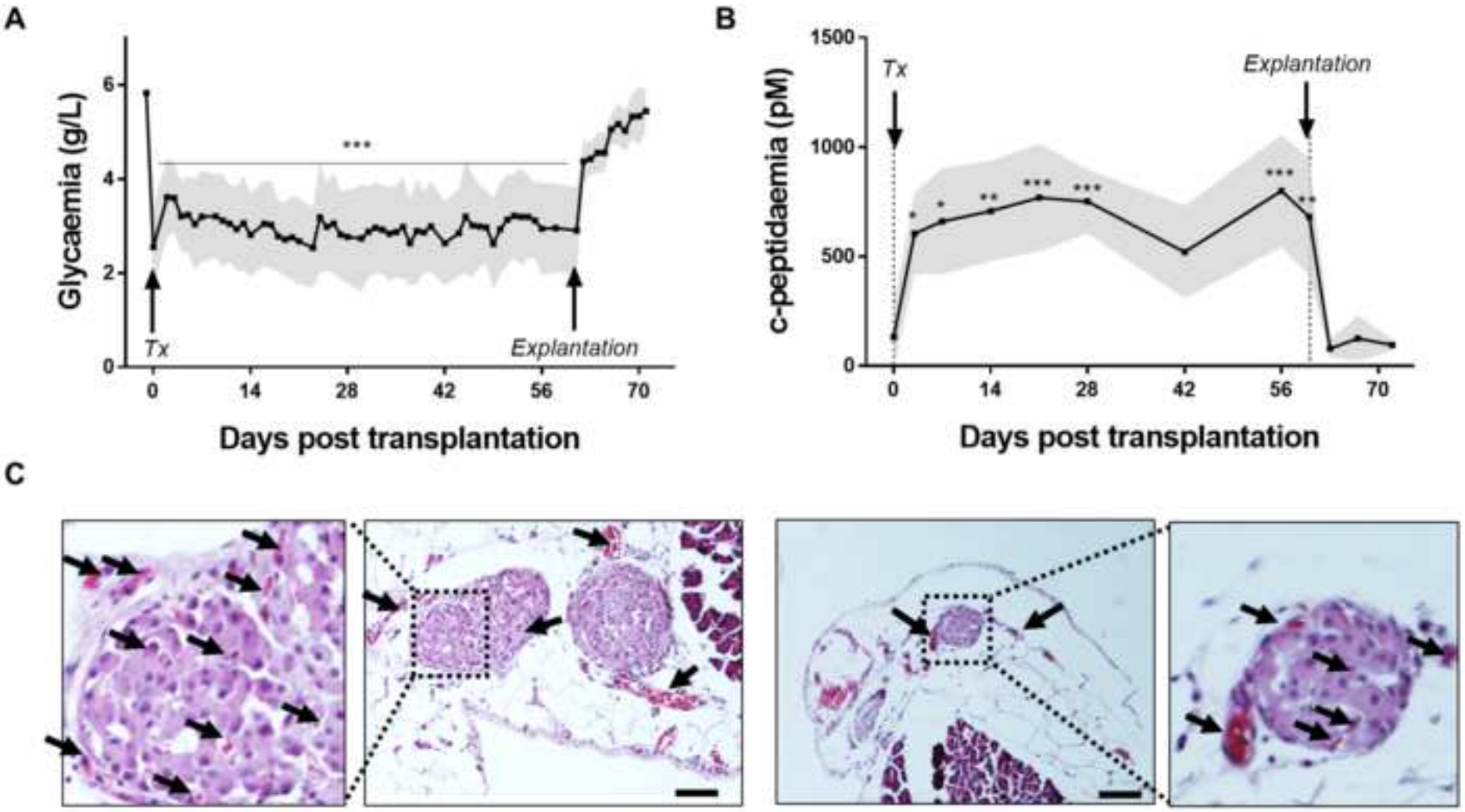


Figure 3



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Alginate (PRONOVA UP LMV)	Novamatrix	4200206	Hydrogel carrier
Atraumatic needle (Blunt)	B.Braun	9180109	
CMRL without FBS	Gibco	11500576	
C-peptide ELISA kit	Mercodia	10-1172-01	
Eosin	Leica Microsystems	3801592E	
Ethilon 4/0isoflo	Ethicon	F2414	Surgical suture
Hematoxylin	Leica Microsystems	3801562E	
Insulin pellets	Linshin	INS-B14	
Isofluorane	Centravet	ISO007	
Lantus (Insulin-Glargin)	Sanofi Adventis	Lantus SoloStar	Long acting insulin
Metacam	Boehringer Ingelheim	MET019	Anti-inflammatory drug
NaCl (for saline 0.9%)	Sigma	10112640	
Needle 26G	TERUMO	050101B	
Oxygen	Linde	2010152	For isoflurane use
Pentobarbital sodique	Vetoquinol	Dolethal	For euthanasia
Steranios 2%	Anios	11764046	
Streptozotocin	Santa-Cruz	SC-200719A	
Syringe – Injekt-F	B.Braun	9166017V	
Trocar & stylet (linshin)	Linshin	G12-SS	For pellet insertion

jove JOURNAL OF VISUALIZED EXPERIMENTS

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Novel in vivo tool for biomaterials validation. Example of intra-omental islet transplantation, hOMING (h-Omental Matrix Islet filliNG).

Author(s): Anaïs Schaschkow1, Carole Mura1, K Bouzakri1, M. Pinget1, Elisa Maillard1.

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: [ ] Standard Access [x] Open Access

Item 2 (check one box):

[x] The Author is NOT a United States government employee.

[ ] The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

[ ] The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	MAILLARD PEDRACINI
Department:	EA 7294 DIATHEC CEED
Institution:	Université de Strasbourg
Article Title:	Novel in vivo tool for biomaterials validation. Example of intra-omental islet transplantation, hOMING (h-Omental Matrix Islet filliNG).
Signature:	
Date:	02/08/2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

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 O_2) 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 function[12]" 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 cm^2 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 graft[11] and allows continuation of intensive insulin therapy and recipient preparation observed in the clinic[12]. 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 drainage[7]."

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 activity[8]."

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 described[10]." 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.