Author’s response to reviews

Title: Do storage solutions protect endothelial function of arterialized vein graft in an experimental rat model?

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RESPONSE TO REVIEWERS

Re: « Do storage solutions protect endothelial function of arterialized vein graft in an experimental rat model?”

Dear Associate Editor,

Thank you very much for the interest about our manuscript as we would like to submit to the Journal of Cardiothoracic Surgery category "Research Article" and entitled “Do storage solutions protect endothelial function of arterialized vein graft in an experimental rat model?”
The manuscript has been reviewed and both reviewers required some explications and changes. Enclose please find our answers, the manuscript with tracked changes colored in red and the final revised manuscript.

Reviewer # 1

Comment 1: “The solutions used in this experimental study are not widely used clinically”

Answer 1: Thank you very much for your comment. Indeed many solutions are used in clinical practice as autologous whole blood at cold (4°C) and warm (28°C), heparinized cardioplegic solution (Bretschneider, St Thomas, Celsior,), heparinized saline solution, buffered solution, lidocaine, nitric oxide (NO), verapamil-nitroglycerin, as you suggested, antispastic solutions, thiobarbituric, human albumin solution, Ringer’s solution or anti-oxydant solutions as GALA and Titrone (Winkler et al. Interact CardioVasc and Thorac Surg, 2016). The practices show that there are currently no ideal preservation solutions. We decided to focus on those three solutions (autologous heparinized blood (AHB), heparinized serum (HS) and GALA solutions) following the publication of Thatte et al. and because GALA is our referent storage solution at University Hospital of Angers since 2005. We used sometimes HS and rarely AHB.

Change 1: This comment is specified in the Introduction section (lines 99-101)

Comment 2: “Without such component (antispastic), the vein graft is usually in spasm...deficiency of the study design”.

Answer 2: Thank you very much for this important comment. The flow-mediated dilatation and myogenic tone of a vein compared to an artery is relatively small because veins do not produce sufficient amounts of NO (Enouri S et al. 2011). Even if there is a spastic component in the graft failure, the principal mechanism is due to an inflammatory response with leukocytes recruitment, SMC migration and proliferation during the acute stage reducing the lumen of the vessel (Ward AO, Atherosclerosis 2017). The response to injury as an important shear-stress produces Reactive Oxygen Species and induces endothelial cell activation (Hallet, 2009). The storage solution should maintain a physiological pH and support endothelial NO production of eNOS substrate. We decided to focus our research on solutions producing an anti-inflammatory effect and protecting the endothelial cells during the storage. Indeed, evidence from ex-vivo studies and animal models is contradictory, and there is a severe lack of clinical studies investigating graft storage solutions.

Change 2: no change but we described the pathophysiology of vein graft failure and the objectives of storage solutions (lines 93-99).

Comment 3: “… is not sure that how well such model represents human CABG surgery”
Answer 3: Thank you very much for this comment. The aim of this model was not to create a GABG model but to evaluate a vein implanted in aortic position and submitted to an important shear-stress. I agree with you that the rat model is not the best to represent human CABG surgery. Big animals are required as dog or pig models (Schachner T et al. EJCTS 2006) but it was not the objective of our experimental model.

Change 3: no change

Comment 4: “It is not sure to use distal aorta…”

Answer 4: Thank you very much for your comment. As I explained in the Answer 2, the vein graft failure is characterized by an inflammatory response with leukocytes recruitment from circulating blood cells. We didn’t consider aorta segment as a control for the vein graft, but we wanted to know if the distal aorta just after the graft which was in the direction of the blood flow, presented inflammatory response and endothelium-layer injury.

Change 4: no change is realized because this comment is explained in the Discussion section (lines 344-347).

Comment 5: “Vein control was GALA-treated but vein graft was treated with which solution?”

Answer 5: Thank you very much for this comment. After the storage in one of the preservation solutions and before arterial implantation, a segment of each vena cava was sampled and stored in PSS corresponding to AHB control vein or HS control vein or GALA vein. For the myography analysis, we had to pool all the data of each group (Vein control = AHB control vein + HS control vein + GALA control vein; Vein graft = AHB vein graft + HS vein graft + GALA vein graft; Aorta = AHB distal aorta + HS distal aorta + GALA distal aorta) due to the small number of graft patency. This is one of the limitations of our study.

Change 5: I specified the control vein definition in the Material and methods section (lines 137-140) and modified the Figure 5 legends (lines 556-563).

Comment 6: “… (the figure legend is wrong) …”

Answer 6: Thank you very much for this comment.

Change 6: I corrected the figure 5 legend (lines 556, 557, 559).

Comment 7: “…Vascular reactivity includes endothelium-dependent and independent-…..”

Answer 7: Thank you very much for this excellent remark. In the figure B, we observed a normal contraction of the aorta to phenylephrine. The contraction for control vein and vein graft
appeared for high dose of phenylephrine. Concerning vascular reactivity, the endothelium-dependent dilatation (Figure 5A) was altered in all groups attesting to endothelial injury. For the endothelium-independent dilatation (Figure 5C), the viability of smooth muscle cells was observed in the vein control group (all preservation solutions maintained the SMC functionality during one hour of storage). In the vein graft and aorta groups, the endothelium-independent dilatation was obtained for high dose of SNP.

Change 7: I added and clarified this remark in the Discussion section (lines 301-309).

Reviewer # 2

Comment 1: “introduction should be shortened and should be more focused”

Answer 1: Thank you very much for this comment. I agree with you, too many details were in the introduction.

Change 1: I deleted lines from 77 to 84 and modified the text in the introduction section.

Comment 2: “postoperative treatment”

Answer 2: Thank you very much to report this important oversight. In fact, we administered antibiotics and analgesic therapies according to the guidelines of the Institutional Animal Care. Concerning the antiplatelet therapy as aspirin, we didn’t use it. In fact, aspirin produces an anti-inflammatory effect which can mask the expected objectives of the storage solutions. Moreover, the literature about vein graft in experimental model does not mention the use of antiplatelet therapy.

Change 2: I added this comment in the Methods section (lines 152-153).

Comment 3: “sample size calculation should be presented”

Answer 3: Thank you very much for this comment. Contrary to the clinical trials, the sample size calculation of our experimental study was built from literature data. In fact, we used the results of the Pinaud’s study (JTCS 2011) realized in our institute. To define the role of the vascular wall in the inflammatory process that may occur with or without pulsatility, they studied resistance arteries functions ex vivo. They measured vascular reactivity, oxidative stress, and inflammation in the arterial wall after 30 and 180 minutes of perfusion with a physiologic salt solution without circulating cells. To show a significant difference between the groups after 180 minutes of perfusion, they used 8 arterial segments from 8 rats per group. Based on this previous study, we estimated that 9 rats per group could show a difference for vascular reactivity and inflammatory response.

Change 3: The sample size calculation is specified in the Methods section (lines 197-200).
Comment 4: “normality criteria should be tested for continuous variables…”

Answer 4: Thank you very much for your comment. I agree with you, it is unclear and wrong in the statistical analysis section. Considering the sample size, non-parametric tests (t-test or Mann-Whitney tests) should and were used in the present study.

Change 4: I corrected this mistake (lines 193-194).

Comment 5: “P=NS should be replaced by the exact number”

Answer 5: Thank you very much for your comment

Change 5: I modified NS by the exact number (lines 213, 216).

Comment 6: “graft thrombosis at 6 weeks should be discussed”

Answer 6: Thank you very much. In fact, we observed a high number of graft thrombosis at 6 weeks among live rats: 2/9 in the AHB group, 5/8 in the HS group and 5/6 in the GALA group. The development of neo-vascularization had limited the risk of limb ischemia. Histological occluded graft analysis showed an important hyperplasia and an intraluminal thrombosis. Results of vein graft patency in murine model were published by Wong et al. (J vasc surg 2014) and showed that the patency rate was 50% at 28 days. The principal experimental studies publishing results at 6 weeks do not detail the patency rates (Sun Q et al. Cardiovasc Res 2012). This deadline is long, the arterialization is obtained at 6 weeks and the vascular remodeling is in the chronic intimal hyperplasia stage leading to the atherosclerosis formation. Our question is: “Can the preservation solutions limit this vascular process?” It is important to not translate these results to the human clinical practice.


Comment 7: “ROS detection… images should be provided”

Answer 7: Thank you very much for this comment.

Change 7: Images were added in the Figure 6 and the comments in the Figure 6 legends.

Comment 8: “references should be presented in the same format”

Answer 8: Thank you very much for this comment. It is true that there are some mistakes in the references section.

Change 8: I corrected and revised all the section.
Comment 9: some relevant papers should be included and discussed.

Answer 9: Thank you very much for your comment. These relevant papers are important and recent.

Change 8: All papers suggested are included and discussed:

• The first paper (ref. 25) lines 252, 254.
• The second paper (ref. 17) line 99
• The third paper (ref. 5) lines line 63
• The fourth paper (ref. 29) lines 292.

Associate Editor

Dear Associate editor,

Thank you very much to have reviewed our paper and for the interest of our work. As you suggested, I shortened the introduction and deleted the molecular explanations. I explained our choice of preservation solutions and detailed the progress of our experimental model.

Hope we answered correctly to all the comments; I remain at your approval to respond to other questions or suggestions.

Sincerely

Dr Olivier Fouquet