Author’s response to reviews

Title: Bovine pericardium retail preserved in glutaraldehyde and used as a vascular patch

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Version: 2 Date: 30 August 2011

Author’s response to reviews: see over
To Editor

Dear Dr.,

Thank you for the attention on our manuscript. As suggested, we have re-written the manuscript in order to improve it. We also thank the referees for their suggestions that really improved the manuscript. We hope the present version can be accepted for publication on this important journal.

With our best wishes.

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To Dr. William Neethling

Dear Dr. William Neethling,

We very much appreciate you for your highly constructive reviews to our submission. We have revised the material to eliminate the issues raised. The added or modified words, phrases, and sentences are in red. We hope the present version can be accepted.

“I. The following typographical errors have been noted in the Background section:
- Page 4, Line 3,”when it was used the autologous vein as arterial substitute.”
  …should be changed to “…when an autologous vein was used as an arterial substitute.”
- Page 4, Line 8, “…in the field of bioprosthetic by introducing the…” should be adjusted or changed.
- Page 4, Line 15, “…..it is observed frequently degeneration of biological tissue…” - word order should be rearranged to “…..degeneration of biological tissue is frequently observed…”.
- Page 5, Line 8, “In clinical practise it has been usually used the smooth side….“
  – word order should be changed to ”In clinical practise the smooth side is usually used with the smooth side facing the lumen,… “”

Answer: We thank the reviewer for the important corrections. We made all corrections suggested by the referee.
“2. In the Method section the following needs attention:
- the product name and the supplier of the pericardium should be mentioned under the Methods section instead of mentioning it in the Discussion section
- Page 5, Line 20, the Animal Ethics Application number should be provided
- Page 6, the supplier of the pharmacological agents should be provided in brackets next to the product name
- Page 6, Line 20, “From each piece it was made 15 cuts merged from 8 to 15 microns.” – Wording should rather be changed to “Fifteen sections (8-15 um) were cut from each tissue sample.”
- Page 7, c), Line 8, “…by assessing extent and thicker.” - the term thicker should probably be “…thickness.”
- Page 7, Line 10, remove the wording “..or so..” – not scientific language.
- Page 8, Line 12, “…in a compact arrange” – should be “….arrangement”

Answer: - We removed the product manufacturer name and inserted it in the Methods section (Methods, 1st paragraph).
- We added the protocol number in the Methods section (Methods, 1st paragraph).
- We included the supplier of the pharmacological agent.
- We replaced the sentence “From each piece it was made 15 cuts merged from 8 to 15 microns.” for “Fifteen sections (8-15 um) were cut from each tissue sample.”.
- We corrected “thicker” to “thickness”.
- We removed the term “or so”.
- We changed “in a compact arrange” for “arrangement”.

“4. Discussion
- Page 9, Line 18, Remove product manufacturer name and insert under Methods section.
- Page 11, Line 18, “….serous surface..” should be rectified, should be serosal surface…”
- Page 12, Line 11, “….the transformation of fibroblast in osteoblasts..” should be adjusted to “…the transformation of fibroblast into osteoblasts.
- Page 12, Line 18, “These authors point out..” should be written in the past tense such as “…pointed out..” while the rest of that same sentence should be altered e.g. “…has a different functional connotation compared to calcification of valvular prostheses,…..”

Answer: - We removed the product manufacturer name and inserted it in the Methods section (Methods, 1st paragraph).
- We changed “serous surface” for “serosal surface”.
- We replaced the sentence “the transformation of fibroblast in osteoblasts” for “the transformation of fibroblast into osteoblasts”.
- We rewrote the sentence “These authors point out...” in order to alter it to the past.

“8. References
- Page 15, Reference No12, - title is incorrect and Journal volume is incorrect.
- Page 16, Reference No 18, - title incorrect.”
Answer: We really appreciate the kind observation of the referee. The corrections were made.

“9. Figure legends
- Figures should be upgraded with detailed arrows or markers to clearly demonstrate and explain legend descriptions. (e.g. Table 1 legend has an asterisk next to the p-value but it is not indicated in the table itself).”

Answer: We thank the reviewer for the suggestion, we added detailed arrows to clearly demonstrate. Tables were rewritten in order to improve it.

“9. Table legends
- Table legends are not explanatory in the current format. P-values should be positioned at the bottom of the table with a clear description to explain the statistical application and importance.”

Answer: We improved the Tables and Tables’ title according to the reviewer’s comment.

“Discretionary Revisions
1. The title needs adjustment e.g. Bovine pericardium retail preserved in glutaraldehyde and used as a vascular patch”

Answer: We made the adjustment required by the reviewer.

To Dr. Anastasios Korossis Sotirios:

Dear Dr. Anastasios Korossis Sotirios,
We thank you very much for your relevant review. Based on your suggestions and critiques, we have made the changes cited below. The added or modified words, phrases, and sentences are in red. We hope the present version can be accepted.

“The methodology, however, is not very well described since the authors do not describe all the test and control groups in the Methods section. Although there is a mention about the peritoneal group in the abstract, there is no mention of this group in the Methods section.”

Answer: We thank the reviewer for the observation. We added in Method section the information regarding the peritoneal group (Methods, 3rd paragraph):

“…facing the lumen and one remnant was juxtaposed to peritoneum”.

“The qualitative data presented is sound but this would have been a much stronger paper if there was quantitative data on the degree of calcification and fibrosis, as well as the mechanical properties of the different test groups.”

Answer: We appreciate the kind suggestion of the reviewer. Unfortunately, we can not perform quantitative analysis with those findings any more. We will use this
suggestion in a future project in which we will work. We request the comprehension of the referee.

“In addition, the authors state that endothelialisation of the patches occurred in the animal model, but the histological results of the explants do not support this.”

Answer: We removed the sentence that states this issue in order to avoid misunderstanding by the reader.

“Although the authors state in the Methods section that they used H&E, Masson’s trichrome and Verhoeff stains, only the results from the H&E and Verhoeff staining of one test group are presented. In general, hematoxylin and eosin is not the best way of investigating tissue calcification.”

Answer: In order to improve this description, we removed Masson’s trichrome method and added the limitation regarding H&E method in Discussion section (Discussion, last paragraph):

“Hematoxylin and eosin is not the best method for investigating tissue calcification and…”

“Moreover, the authors state that the explants presented “high tensile resistance”, but they did not conduct any quantitative biomechanical testing to substantiate this.”

Answer: We agree with the referee, we removed the sentence according to the reviewer’s comment.

“The limitations of the work are not clearly stated; a section clearly stating the limitations of the study needs to included at the end of the manuscript.”

Answer: We included in this new version the limitations of our study (Discussion, last paragraph):

“Our investigation presents some points that should be addressed: we did not perform blood analysis, i.e., cholesterol, triglycerides and blood glucose levels. However, we aimed to focus only on calcification. Hematoxylin and eosin is not the best method for investigating tissue calcification and it was not quantified proteins related to fibrosis. On the other hand, this is the first study to investigate the utilization of different faces of bovine pericardium conserved in glutaraldehyde as a vascular patch. The glutaraldehyde treatment, the resulting increased stiffness of the treated tissue and the presence of fixed cellular material in the tissue are all factors contributing to the calcification of cardiovascular implants. We did not perform microbiological investigation of the patch. We suggest this procedure to future studies. The time the bovine patch stayed in the circulation was not constant, possibly this factor may be a bias for our findings.”

“The Discussion is well balanced but the Conclusions are based on circumstantial/qualitative evidence and need further substantiation.”

Answer: We changed the conclusion based on the reviewer’s comment:
“When turned to the inner portion of the vessel, the rough face of the remnant had a lower dilatation in relation to the adjacent aorta and a better quality of endothelium layer and there was no difference between intensity and/or incidence of graft calcification. Therefore, we suggest the surgeons to use the smooth face.”

“In addition, the authors present in the discussion some possible explanation for their findings, and they attribute the calcification of all their explanted grafts to the predisposition of the young animals to calcification. However, the glutaraldehyde treatment itself, the resulting increased stiffness of the treated tissue, and the presence of fixed cellular material in the tissue, are all factors contributing to the calcification of cardiovascular implants. These factors have not been addressed adequately in the Discussion.”

Answer: We thank the reviewer for the important comment. We added this limitation in the Discussion (Discussion, last paragraph):

“The glutaraldehyde treatment, the resulting increased stiffness of the treated tissue and the presence of fixed cellular material in the tissue are all factors contributing to the calcification of cardiovascular implants. We did not perform microbiological investigation of the patch. We suggest this procedure to future studies.”

“The manuscript lacks an adequate quality in terms of the writing, and major revision needs to be undertaken to improve the scientific language used.”

Answer: We thank the referee for the comment, English Grammar and Spelling were extensively reviewed.

“Page #5; § Methods: We used bovine pericardium produced commercially. Please give more information about the product.”

Answer: We added product’s information on this sentence (Methods, 1st paragraph):

“The material used in our study was produced by Braile Biomédica® industry.”

“Page #5; § Methods: ......glutaraldehyde, obtained from the Brazilian industry. Please give more information about the chemicals used.”

Answer: We added the name of the industry: “...obtained from the Gravataí industry.”

“Page #10; § Discussion: Furthermore, we observed that the bovine pericardium patch presented good resistance. What do the authors mean here? Resistance to what?”

Answer: We thank the reviewer for the observation. We removed this sentence in order to avoid misunderstanding.

“Page #17; § Figure Legends; Figure 3: Please revise the group references; FR? FL?”
Answer: We really appreciate the observation of the referee. We replaced FR and FL for RF and SF, respectively. That was a mistake due to the translation.

“Page #24; § Figure 1: Please use arrows to indicate the position of the implants. 
Page #25; § Figure 2: Please use arrows to indicate the position of the implants. 
Page #26; § Figure 3: Please use arrows to indicate the location of calcification.”

Answer: We added arrows in the figures and added this information on figure legends.

To Dr. Thedosios Bisdas:

Dear Dr. Thedosios Bisdas,

We are very thankful for your helpful revision. We have reviewed the material in order to eliminate the issues raised. The added or modified words, phrases, and sentences are in red. We hope the present version can be accepted.

“1. Bovine pericardium is mostly implanted for the vascular reconstruction in the femoral or carotid artery as patchplasty. Especially in the carotid artery, do you propose the use of rough face?”

Answer: We appreciate the referee for the kind comment and suggestion. Based on our data, we can not confirm the use of the rough face in the carotid artery. We suggest future studies to investigate this possibility. We added a new paragraph regarding this issue (Discussion, 10th paragraph):

“Bovine pericardium is also implanted for the vascular reconstruction in the femoral or carotid artery as patchplasty and venous patch angioplasty [27]. Moreover, the bovine pericardium is not only used in cardiovascular surgery but also in hernia [28] or thoracic surgery [29]. Based on our data, we confirm the use of the rough face in those types of surgery. We propose future studies to investigate this possibility.”

“2. You choose a bovine pericardium produced by Braile Biomedica. However, there are several industries that produce the same patch. The more interesting approach is from the industry Synovis producing the Vascu-Guard. In order to reduce the prevalence of calcification, they follow the so-called 'Apex-Processing'. Briefly, in this process, the levels of residual glutaraldehyde are below the limits of detection by the sophisticated analytical methods now available (<0.5 ppm) and products undergoing Apex-Processing have levels of cellularity that are four times lower than a variety of competitive materials including products 'conventionally treated'. Please consider to add these data in your discussion (http://www.obex.co.nz/Shared/Documents/Technical%20Review%20Apex%20Processing.pdf).”

“3. Besides, the calcification content in bovine pericardium with Apex-processing amounted to 0.4 microgram/mm in comparison to 24 microgram/mm for conventional bovine pericardium. Is not possible to have such a measure of calcification instead of crosses and three categories. Than your data will be more comparable.”
Answer: We are more than happy to discuss those important data and learn about this path. We added a new paragraph that cites this issue (Discussion, 8th paragraph):

“We used a bovine pericardium produced by Braile Biomedica. There are several industries that produce the same patch. One may wonder that one of the most interesting approaches come from the industry Synovis producing the Vascu-Guard [30]. In order to reduce the prevalence of calcification, they follow the Apex-Processing. Briefly, in this process, the levels of residual glutaraldehyde are below the limits of detection by the sophisticated analytical methods now available (<0.5 ppm) and products undergoing Apex-Processing have levels of cellularity that are four times lower than a variety of competitive materials including products conventionally treated.”

“4. Did you perform any microbiological investigation of the patch? Please describe the results of the anatomopathological investigation in the one pig with wound infection.”

Answer: We thank the reviewer for the important suggestion. Unfortunately, we did not perform microbiological investigation of the patch. We added this limitation in the last paragraph of the Discussion section and we suggested it for future studies (Discussion, last paragraph):

“We did not perform microbiological investigation of the patch. We suggest this procedure to future studies.”

“5. Which references did you use for the definition of calcification, integration tissue, internal apposition fibrosis etc?”

Answer: We thank the reviewer for the relevant observation. We added the following references:


“6. Please provide more data about the cellular structure of the patches in each group after explantation.”

Answer: We added a new paragraph in the Results section that provides more details regarding the structure (Results, last paragraph):
“The anatomic and pathologic evaluation showed that of all parts of the bovine pericardium was conserved. Moreover, we found a superposition of a fibroelastic dense connective tissue collagen fibers on the face found facing the lumen of the vessel, which was modeled in a compact arrangement and arranged parallel to the surface, similar to dense connective tissue modeled. There were no degenerative, inflammatory or metaplastic diseases.”

“6. Please make a more clear conclusion. Should the surgeons use the rough or the smooth face? If you are not able to provide such a conclusion, I propose to design a table with the results of all studies investigating the same topic. By this way, the reader will have a better overview to make the decision for the clinical practice.”

Answer: We thank the reviewer for the comment that really helped us to make the conclusion clearer to the reader. We remade conclusion (Conclusion section):

“When turned to the inner portion of the vessel, the rough face of the remnant had a lower dilatation in relation to the adjacent aorta and a better quality of endothelium layer and was not showed difference between intensity and/or incidence of graft calcification. Therefore, we suggest the surgeons to use the smooth face.”

“I. You have implanted 3 remnants in each pig (2 in the abdominal aorta and 1 was juxtaposed to peritoneum). Please change this in the abstract (Fourteen young pigs...replace abdominal aorta) and in the patients and methods (In each animal we implanted...facing the lumen).”

Answer: According to the reviewer observation, we remade this sentence in the Abstract and in the Methods, section:

“We implanted three remnants in each pig, two in the abdominal aorta and one was juxtaposed to peritoneum.”

“We implanted three remnants in each pig, two in the abdominal aorta and one was juxtaposed to peritoneum, with a smooth face (SF) and the other with the rough face (RF)...”

“2. Please replace the crosses with degress (+++ -> 3rd degree)”

Answer: We replaced + for 1st degree, ++ for 2nd degree and +++ for 3rd degree.

“I. The bovine pericardium is not used only in cardiovascular surgery but also in general- (hernia) or thoracic surgery (chest defects). Which is your recommendation in those cases according to your results? The answer could be added in your discussion to improve the clinical relevance of your manuscript.”

Answer: We really appreciate the referee for the kind observation, which helped us to extensively improve the discussion. We can not confirm the use of the rough face in those cases, however, we may stimulate future studies. We inserted a new paragraph regarding this matter (Discussion, 10th paragraph):
“Bovine pericardium is also implanted for the vascular reconstruction in the femoral or carotid artery as patchplasty and venous patch angioplasty [27]. Moreover, the bovine pericardium is not only used in cardiovascular surgery but also in hernia [28] or thoracic surgery [29]. Based on our data, we confirm the use of the rough face in those types of surgery. We propose future studies to investigate this possibility.”

To Dr. Miltiadis Matsagkas:

Dear Dr. Miltiadis Matsagkas,

We are glad for your relevant revision that really helped us to improve the manuscript. All added or modified words, phrases, and sentences are in red. We hope the present version can be accepted.

“1. Background: It is very bulky repeating a lot of things and in some aspects looking as a discussion section. It has to be cut to at least the half size.”

Answer: We thank the referee for the comment. Based on his suggestion, we shortened Background section.

“2. Methods: A clear description of the experiment has to be given. Important information is missing. For example how many months these experiments lasted in total? Was the operation made by the same surgical team? Why was such a big variety in the time the pigs were sacrificed? Were there subgroups of 4 months and others for 8 months follow-up? Otherwise the time the bovine patch stayed in the circulation is not constant and this may bias the results”

Answer: We really appreciate the comments of the reviewer that helped us to improve the methods and discussion sections. The variety in the time the pigs were scarified is due to their different times of recovery. We added a new paragraph that indicates the total period of the experiments and regarding the surgical team (Methods, 12th paragraph):

“The experiments lasted a total of 14 months and the surgical procedures were performed by the same surgical team.”

With respect to the other questions, we added those limitation in Discussion section (Discussion, last paragraph):

“The time the bovine patch stayed in the circulation was not constant, possibly this factor may be a bias for our findings.”

“3. Methods: There has been an attempt to evaluate the results in a quantitative manner but of course this is not pure quantification, and is subjected to systemic bias according to the reader. In my opinion there had to be at least two readers of the results and the inter-observer variation to be calculated.”
Answer: We thank the reviewer for the kind comments that helped us to remember this important issue. We added this information in the Methods section (Methods, 12th paragraph):

“There were two readers for each data.”

“4. Discussion: It is very bulky causing some confusion to the reader. I suggest cutting it a lot and mainly focusing on the most important findings of the study, thus the differences between the two graft surfaces.”

Answer: According to the referee’s suggestion, we cut some parts in the discussion which were not related to the difference between the two graft surfaces.

“1. The background has to be revised; it gives inadequate information about the study.”

Answer: The background was extensively revised based on the referee comment.

“2. Methods: line 1: the manufacturer of the patch has to be stated clearly here and not in the discussion.”

Answer: We added product’s information on this sentence (Methods, 1st paragraph):

“The material used in our study was produced by Braile Biomédica® industry.”

“3. Results: the results about the patch sutured in the peritoneum are not even mentioned in the results section. There is only some statement in the discussion section”

Answer: We thank the reviewer for the suggestion. We added a paragraph that briefly discusses this finding (Discussion, 9th):

“According to our data, there was no calcification in the retail implanted in the peritoneum in almost the half of the animals. Peritoneal calcification is a rare condition developed in uremic patients on continuous ambulatory peritoneal dialysis. Once peritoneal calcification is detected, it is essential to assess whether encapsulating peritoneal sclerosis develops [26].”

“1. It seems better to put some arrows in the figures, thus making it easier for the reader to understand them.”

Answer: Based on the reviewer suggestion we added arrows in the figures.