Reviewer Assessment

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Dehydration improves biomechanical strength of bioartificial vascular graft material and allows its long-term storage

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Reviewers’ Comments to Original Submission

Reviewer 1: Balazs Gasz
May 21, 2018

Reviewer Recommendation Term: Accept with Minor Revision
Overall Reviewer Manuscript Rating: 70

Custom Review Questions
Is the subject area appropriate for you? 4
Does the title clearly reflect the paper’s content? 4
Does the abstract clearly reflect the paper’s content? 4
Do the keywords clearly reflect the paper’s content? 5 - High/Yes
Does the introduction present the problem clearly? 3
Are the results/conclusions justified? 4
How comprehensive and up-to-date is the subject matter presented? 4
How adequate is the data presentation? 5 - High/Yes
Are units and terminology used correctly? 5 - High/Yes
Is the number of cases adequate? 5 - High/Yes
Are the experimental methods/clinical studies adequate? 4
Is the length appropriate in relation to the content? 4
Does the reader get new insights from the article? 4
Please rate the practical significance. 4
Please rate the accuracy of methods. 3
Please rate the statistical evaluation and quality control. 5 - High/Yes
Please rate the appropriateness of the figures and tables. 3
Please rate the appropriateness of the references. 4
Please evaluate the writing style and use of language. 4
Please judge the overall scientific quality of the manuscript. 4
Are you willing to review the revision of this manuscript? Yes

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Comments to Authors:
In the present paper authors evaluate the dehydration and storage of fibrin tubes applied as vascular small-diameter graft. During the study the effect of dehydration process and long-term storage has been evaluated. In previous studies the team described the method of fibrin vascular graft preparation and its function in experimental model, with very promising results. According to the results and conclusion of the study it can be summarized that the method presented by authors enable construction, dehydration and storages of small diameter vascular grafts for 6 months, whereas after 12 mounts of storage period the majority of grafts lost their integrity (5 of 10 was intact according to the observation methods).

The study has crucial importance and - after clarifying some further questions - the method has potential application as a product of clinical use in yet unsolved area. The investigations are well designed, the statistical analysis and the presentation of the results are correct. The most important deficiency of the paper is the simplified methodology for analysis of vascular graft tubes. Several, more impressive and comprehensive analysis of the tube-structure, like micro CT, electron microscopy, permeability or calorimetry may increase the impact of the study and also may help to understand the processes occurring during storage and dehydration method. On the other hand, in the present form, the methods demonstrated in the article are eligible for conclusions and performing the first evaluations.

It can be the trouble with downloading, but tables cannot be reach in the manuscript. The completion of manuscript with tables is essential for understanding.

Minor concerns and questions:
* The introduction section is far too long and descriptive. Reduction of introduction section is suggested for being more focused to the main topic of the paper. Focusing on problem of long-term storage of vessel tubes and short review of the structural changes during the dehydration and storage may help to better understanding for and interest of the readers.
* Although the wall thickness was thoroughly measured in the groups, was the efficacy and reproducibility of dehydration process assessed by measuring the amount of water-loss? Have any study, including any preliminary study performed to measure the weight change of tubes during the dehydration and rehydration? And how was it changed during shorter and longer-term storage?
* It was described, that the devices where sterile during the procedure. How can the vascular tubes sterilized and was there any further assessment to clarify the sterility of the grafts?
* On figure 3 it is described and visible, that the tubes after 12 months can lost integrity and fissures occur. How was the integrity of the tubes after 6 months evaluated, in detail? Can any permeability analysis assess the smaller than visible fissures or morphological changes? Is any data available about the leakage/permeability of the tubes in the 4 group?
* Discussion section is suggested to be completed with one-two sentences about comparison with further TE procedures and decellularized models and biomechanical properties.

In conclusion the paper describes novelties with potentially high clinical impact, after the completion of these minor deficiencies the paper might be able for publication.

Reviewer 2: Dirk Wilhelm
May 16, 2018

**Reviewer Recommendation Term:** Revise with Major Modification

**Overall Reviewer Manuscript Rating:** 55

**Custom Review Questions Response**

| Question                                                                 | Response |
|-------------------------------------------------------------------------|----------|
| Is the subject area appropriate for you?                                | 2        |
| Does the title clearly reflect the paper’s content?                    | 3        |
| Does the abstract clearly reflect the paper’s content?                 | 3        |
| Do the keywords clearly reflect the paper’s content?                   | 3        |
| Does the introduction present the problem clearly?                     | 5 - High/Yes |
| Are the results/conclusions justified?                                 | 4        |
| How comprehensive and up-to-date is the subject matter presented?      | 4        |
| How adequate is the data presentation?                                 | 3        |
| Are units and terminology used correctly?                              | 5 - High/Yes |
| Is the number of cases adequate?                                       | 4        |
| Are the experimental methods/clinical studies adequate?                | 4        |
| Is the length appropriate in relation to the content?                  | 1 - Low/No |
| Does the reader get new insights from the article?                     | 3        |
| Please rate the practical significance.                                | 4        |
| Please rate the accuracy of methods.                                   | 4        |
| Please rate the statistical evaluation and quality control.            | 2        |
Please rate the appropriateness of the figures and tables. 3
Please rate the appropriateness of the references. 4
Please evaluate the writing style and use of language. 3
Please judge the overall scientific quality of the manuscript. 3
Are you willing to review the revision of this manuscript? Yes

Comments to Authors:
Dear authors, thank you for submitting your manuscript upon dehydration of vascular grafts to the ISS. The topic herein is of relevance and the problem described well addressed. Also the applied methodology and analysis are well performed, however some issues of the article require revision. First of all, the introduction is much too long and not to the point, as more focused to the overall problem of vascular grafts than on the topic of dehydration and storage of bio implants. The same attributes to the discussion, that is addressing the results of the study only in part and that omits relevant aspects. E.g. how do you explain the gain in strength after dehydration/rehydration that remains over a period of 6 months and is then lost? Why is the wall thickness of the dehydrated (and rehydrated?) graft thicker than before this process. Do you expect the graft being integrated and remodeled in the same matter as in a non-dehydrated implant? Is the concept of rehydration overnight practicable in clinical routine and necessary at all? Please revise the discussion accordingly. Some other questions also arise during reviewing that also require revision. Most importantly, please specify the dehydration process more in detail, or was it performed really in such manner? Was there a change of temperature or reduction of the humidity of air, as typical in cryoconservation? Statistical analysis do not include comparative analysis and are only descriptive. Furthermore, a table that visualizes all results in comparison would be of highest interest. Finally, there are some linguistic and spelling errors among the text that need to be corrected.

The topic presented in the article is relevant and the methods applied appropriate, however the article is unfocused and includes some irrelevant information while omitting interesting aspects on the other hand. Accordingly, I would recommend thorough revision of the article.

Authors’ Response to Reviewer Comments

Jun 06, 2018

Dear Reviewers,
Thank you for reviewing our manuscript. We have revised the manuscript according to your comments. Namely the sections introduction and discussion were completely revised and shortened. Changes in the manuscript are underlined.

Introduction
Reviewer 1
The introduction section is far too long and descriptive. Reduction of introduction section is suggested for being more focused to the main topic of the paper. Focusing on problem of long-term storage of vessel tubes and short review of the structural changes during the dehydration and storage may help to better understanding for and interest of the readers.

Reviewer 2
First of all, the introduction is much too long and not to the point, as more focused to the overall problem of vascular grafts than on the topic of dehydration and storage of bio implants.
- We have completely rewritten the introduction section and shortened it by one third. Figure 1 has been removed.

Materials and methods
Reviewer 1
It was described, that the devices where sterile during the procedure. How can the vascular tubes sterilized and was there any further assessment to clarify the sterility of the grafts?
- A subsequent sterilization of the fibrin tubes is an important feature for a potential later clinical use and is planned to be evaluated in further experiments. In the presented study fibrin tubes were generated from sterile compounds in a sterile mould. But, sterility of the fibrin tubes after dehydration process and storage was not proven. Contamination of the fibrin tubes might one reason for the observed loss of stability and integrity during storage. We have added this point to the discussion section.

Reviewer 2
Most importantly, please specify the dehydration process more in detail, or was it performed really in such manner? Was there a change of temperature or reduction of the humidity of air, as typical in cryoconservation?
- The dehydration process was done as described under room temperature without changes of the environmental temperature (we have added this point to the description). Changes in the temperature would be needed in a way, if cryoconservation of vacuum drying would have been done. But, in this admittedly simple experimental setup, fibrin tubes were only dried in dry air. To achieve an environment with dry air, humidity was reduced only by addition of sodium chloride to the flask. We have referred to this relatively simple experimental set-up in the manuscript (materials and methods and discussion). There is no question, that this process as well as the further storage can be optimized in further studies. The purpose of this study was to examine the extent to which dehydration of the fibrin matrix affects its properties. And since the well-known methods that allow storage of a biological matrix also work with dehydration, we have evaluated the possibilities of storage of the dehydrated fibrin matrix. This can certainly be optimized. We have added this point in the discussion.

Results

Reviewer 1
The most important deficiency of the paper is the simplified methodology for analysis of vascular graft tubes. Several, more impressive and comprehensive analysis of the tube-structure, like micro CT, electron microscopy, permeability or calorimetry may increase the impact of the study and also may help to understand the processes occurring during storage and dehydration method. On the other hand, in the present form, the methods demonstrated in the article are eligible for conclusions and performing the first evaluations.
- We have added electron microscopic scans to the figures. Unfortunately, we did not make these scans from specimens of all groups for comparative studies. MRT-studies have so far not brought the desired information due to an insufficient at present. We are still working on this issue.

Reviewer 1
Although the wall thickness was thoroughly measured in the groups, was the efficacy and reproducibility of dehydration process assessed by measuring the amount of water-loss? Have any study, including any preliminary study performed to measure the weight change of tubes during the dehydration and rehydration? And how was it changed during shorter and longer-term storage?
- We did not weigh the fibrin tubes. This is in our opinion a defective method, because some water adheres to the segments in the state immediately after their generation namely in the lumen. We have chosen the calculation of the volume of the cured fibrin/ the volume of the wall of the segments itself, for estimation of water less/ the degree of compaction.

On figure 3 it is described and visible, that the tubes after 12 months can lost integrity and fissures occur. How was the integrity of the tubes after 6 months evaluated, in detail? Can any permeability analysis assess the smaller than visible fissures or morphological changes? Is any data available about the leakage/permeability of the tubes in the 4 group?
- Integrity of the fibrin tubes after storage was evaluated by the measurement of burst strength. If pressure could be built up during the filling with fluid, the fibrin tubes were defined as intact.
In 5 of 9 fibrin tubes, which were stored for 12 months, during measurement of the burst strength no pressure could be built up due to fissures in the tubes.
These points were added to the manuscript.

Reviewer 2
Statistical analysis do not include comparative analysis and are only descriptive. Furthermore, a table that visualizes all results in comparison would be of highest interest.
- We revised the tables to address this point. Significant differences are marked now in figure 5.

Discussion

Reviewer 1
Discussion section is suggested to be completed with one-two sentences about comparison with further TE procedures and decellularized models and biomechanical properties.
- We added this point to the discussion.

Reviewer 2
The same attributes to the discussion, that is adressing the results of the study only in part and that omits relevant aspects. E.g. how do you explain the gain in strength after dehydration/rehydration that remains over a period of 6 months and is then lost?
- We have completed revised the discussion section and added the points mentioned by the reviewers.

Why is the wall thickness of the dehydrated (and rehydrated?) graft thicker than before this process?
- During dehydration process a dilator was placed inside the tubes to prevent lumen collapse, to which the fibrin attached. This attachment kept the length of the fibrin tubes at 100 mm. Thus, shrinkage was limited to the diameter given by the size of the dilator inside (2.7 mm).
While the wall thickness increased after dehydration, the total volume of the cured fibrin in the wall of the segments decreased. We described this in more detail in the manuscript to clarify this point to the.

Do you expect the graft being integrated and remodeled in the same matter as in a non-dehydrated implant?
- While fibrin has been proven to be an ideal matrix for the seeding with different cell types and allows their ingrowth and three-dimensional arrangement, further in vivo experiments must prove the suitability of the dehydrated fibrin tubes. To what extent the decrease of the space between the fibrils (pore size) has an effect on the adhesion and migration of cells as a decisive step of remodelling cannot currently be estimated. Presumably, only the planned further in vivo experiments can answer the question as to whether remodelling is delayed or even impaired and whether the compaction of the surface may even have a positive effect on a decrease in thrombogenicity.

Is the concept of rehydration overnight practicable in clinical routine and necessary at all? Please revise the discussion accordingly.
- A rehydration of the dehydrated fibrin tubes for 1 hour is sufficient for the further use. We kept the fibrin tubes in in sodium chloride solution (0.9%) overnight to ensure that the rehydration was completed before the further characterization and that there would be no potential additional swelling of the matrix for example after a potential implantation in vivo, which might decrease the measured biomechanical properties.

**Tables**

**Reviewer 1**

It can be the trouble with downloading, but tables cannot be reach in the manuscript. The completion of manuscript with tables is essential for understanding.

**Reviewer 2**

A table that visualizes all results in comparison would be of highest interest.
- All tables are summed up in one table now.

**Reviewers’ Comments to Revision**

**Reviewer 1: Balazs Gasz**

Jun 11, 2018

| Reviewer Recommendation Term: | Accept |
|-------------------------------|--------|
| Overall Reviewer Manuscript Rating: | 75 |

**Custom Review Questions**

| Question                                                                 | Response       |
|-------------------------------------------------------------------------|----------------|
| Is the subject area appropriate for you?                                | 4              |
| Does the title clearly reflect the paper’s content?                     | 4              |
| Does the abstract clearly reflect the paper’s content?                  | 5 - High/Yes   |
| Do the keywords clearly reflect the paper’s content?                    | 5 - High/Yes   |
| Does the introduction present the problem clearly?                      | 3              |
| Are the results/conclusions justified?                                  | 4              |
| How comprehensive and up-to-date is the subject matter presented?       | 4              |
| How adequate is the data presentation?                                  | 4              |
| Are units and terminology used correctly?                               | 5 - High/Yes   |
| Is the number of cases adequate?                                        | 4              |
| Are the experimental methods/clinical studies adequate?                 | 4              |
| Is the length appropriate in relation to the content?                   | 4              |
| Does the reader get new insights from the article?                      | 4              |
| Please rate the practical significance.                                 | 4              |
| Please rate the accuracy of methods.                                    | 3              |
| Please rate the statistical evaluation and quality control.             | 5 - High/Yes   |
| Please rate the appropriateness of the figures and tables.              | 4              |
Please rate the appropriateness of the references. 4
Please evaluate the writing style and use of language. 4
Please judge the overall scientific quality of the manuscript. 4
Are you willing to review the revision of this manuscript? Yes

Comments to Authors:
According to the changes performed in the manuscript, I suggest the paper for publication in ISS.

Reviewer 2: Dirk Wilhelm
Jun 14, 2018

Reviewer Recommendation Term: Accept
Overall Reviewer Manuscript Rating: 75

Custom Review Questions Response
Is the subject area appropriate for you? 3
Does the title clearly reflect the paper’s content? 4
Does the abstract clearly reflect the paper’s content? 4
Do the keywords clearly reflect the paper’s content? 4
Does the introduction present the problem clearly? 4
Are the results/conclusions justified? 4
How comprehensive and up-to-date is the subject matter presented? 4
How adequate is the data presentation? 4
Are units and terminology used correctly? 4
Is the number of cases adequate? 5 - High/Yes
Are the experimental methods/clinical studies adequate? 4
Is the length appropriate in relation to the content? 3
Does the reader get new insights from the article? 4
Please rate the practical significance. 4
Please rate the accuracy of methods. 4
Please rate the statistical evaluation and quality control. 4
Please rate the appropriateness of the figures and tables. 4
Please rate the appropriateness of the references. 5 - High/Yes
Please evaluate the writing style and use of language. 5 - High/Yes
Please judge the overall scientific quality of the manuscript. 4
Are you willing to review the revision of this manuscript? Yes

Comments to Authors:
Dear authors, thank you for revising the manuscript according to the reviewers comments. Accordingly, the manuscript has improved in clarity and significance so I would support its publication.