Author's response to reviews

Title: The feasibility of ureteral tissue engineering using autologous veins: an orthotopic animal model with long term results

Authors:

Oliver Engel (o.engel@uke.de)
Robert de Petriconi (robert.petriconi@uniklinik-ulm.de)
Björn G Volkmer (bioern.volkmer@klinikum-kassel.de)
Kilian M Gust (kilian.gust@arcor.de)
Jens Mani (jens.mani@kgu.de)
Axel Haferkamp (axel.haferkamp@kgu.de)
Richard E Hautmann (richard.hautmann@uni-ulm.de)
Georg C Bartsch (georg_bartsch@yahoo.com)

Version: 3
Date: 28 September 2014

Author's response to reviews: see over
Dear Prof. Brummelte,

Thank you for giving us the opportunity to submit our revised manuscript:
MS: 1815564998130654
The feasibility of ureteral tissue engineering using autologous veins: an orthotopic animal model with long term results
Oliver Engel Robert de Petriconi Bjoern G Volkmer Kilian M Gust Jens Mani Axel Haferkamp Richard E Hautmann and Georg C Bartsch

We adapted the manuscript to the reviewers’ suggestions as good as possible.

Reviewer: Jaqueline C Rinaldi
Reviewer’s report:
Minor Essential Revisions
Comments:
The manuscript entitled "The feasibility of ureteral tissue engineering using autologous veins: an orthotopic animal model with long term results" describes the use of tissue engineered constructs for ureteral replacement in a long term orthotopic minipig model. Overall the data presented in this manuscript is very interesting and supports the venous grafts may be a potential source for ureteral reconstruction. However, there are a few concerns.

1) I suggest reviewing English and text format, for example: A) Title, 1st line: “feasibility” instead of “feasibility”; B) Abstract/Background, 2nd line: “in vivo” and “in vitro” are not in italic; C) Results, 3rd line: “In week 24 week one animal”.

Answer to the reviewer: Thank you for mentioning the errors, we corrected them and reviewed the manuscript for typing and grammar errors thoroughly.

2) Figure (6): I suggest describing the photomicrography or put letters to identify the tissues. You are missing some important details of the tissue morphology.

Answer to the reviewer: Thank you, we included this into the figure and the figure legends. To be able to adapt this figure, we had to change the format from Word to pdf.

Changed passage in the figure legend:
In the tissue engineered ureter an urothelial lining (U) is evident on top of the venous scaffold (V) (left row) while in the unseeded grafts no urothelial lining is noticeable. In the third row the typical ureteral histologic architecture with urothelium (U), submucosal layer (SM), and muscular layer (M) is recognisable. While the H&E
staining reveals the general architecture, in the Trichrom-Masson staining the low content of collagen (blue color) in all tissues representing the low grade of scar tissue formation is noticeable.

3) Figure (7): Indicate the immunostained cells to be clear the immunoreactivity of the tissue. If it is not the case (there was no reaction into the tissue, I suggest write this information in the legend). Did the authors have done positive and negative control of this reactions?

Answer to the reviewer: We agree and included a sentence stating that the reaction was specific and not reactive. We did not mention this in the text, but all immunohistochemistry has been established with corresponding positive and negative controls.

Changed passage:

Specific Immunostained cells were clearly distinguishable from immunoreactivity of investigated tissue.

4) I would like the authors discuss their comment: “With low sample number it is hard to tell, whether tissue engineered segments even have a disadvantage compared with unseeded constructs in long-term follow up” (Discussion, 3rd paragraph, 8nd line) and “These findings suggested that this method may be a feasible method to substitute the ureteral wall” (Discussion, 6nd paragraph, 4nd line) to highlight the main contributions with their manuscript.

Answer to the reviewer: We hope we understood this the right way and rephrased that passages accordingly:

Concerning the necessity of a urothelial layer, earlier studies have determined that short gaps are closed by ingrowing urothelium, but longer distances lack an urothelial lining. Dorin et al. showed that the maximum distance for tissue regeneration is 0.5 cm [18]. The urothelial layer is reported to essential to protect the neoureter from aggressive urinary components, which may lead to extensive scarring and consecutive stenosis. This hypothesis may be supported with the 100% patency rate in the short-term (12 weeks). Still, we have to acknowledge that in our study three out of four tissue engineered constructs failed in the long term (24 and 48 weeks), while unseeded venous grafts performed better (only one out of four ureters failed). For
drawing the conclusion that urothelial cell seeding is not necessary and that the endothelial cells on the native autologous veins are sufficient to reconstruct the ureter the sample size of this cohort is too small. The risk for a surgeon dependent bias of result is low; all contributing surgeons have high experience in reconstructive urologic procedures.

**Level of interest:** An article of importance in its field  
**Quality of written English:** Needs some language corrections before being published  
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Reviewer:** Yuanyuan Zhang

**Reviewer's report:**

1. What is the rational of using urothelial cell-seeded vein as a scaffold? The reason that vein with non-cell seeding achieved better result might be due to well-preserved entire layer of endothelial cells on the lumen side. A fresh vein vessel alone might be better in ureter regeneration.

   **Answer to the reviewer:** We agree with the reviewer, but think that with the small sample size in this study it would be too aggressive to draw the conclusion to use fresh autologous veins without urothelial cell seeding for ureteral reconstruction. The rationale to seed urothelial cells on top of the veins is explained by the findings of the current literature (as described in the discussion, page 9) that only urothelial cell seeded scaffolds are able to withstand the aggressive components of the urine. We agree that it may be that endothelial cells may be sufficient to protect the connective tissue. It may be more feasible to use native and fresh venous tissue for ureteral tissue reconstruction. But as said, we believe the results are not clear enough to draw this conclusion.

2. It would be helpful if information about jugular vein graft could be provided, such as:

   #whether were veins decellularized and why was it called as vein matrix?

   **Answer to the reviewer:** Thank you for this remark. The veins were not decellularized. We included this in the methods section. We changed all venous matrix to veins. (page 13)

**Changed passage:**

The veins were used in a native fashion and were not processed chemically or enzymatically.
#Why were jugular vein used, were the vein graft sizes enough for the onlay patch of the ureter?

*Answer to the reviewer:* We were not able to find a vein of higher diameter in the porcine model, which may be used in an autologous fashion, without causing a high rate of morbidity to the animal. The vein size was far enough to perform the ureteral patch.

#Are there any native endothelial and smooth muscle cells left after vein are cultured in vitro prior to implantation and months after implanted in vivo as well?

*Answer to the reviewer:* Yes there are. This was shown in earlier studies [2]. We referenced this in the background section, line 13 and in the discussion section, line 9.

3. Please describe the gross observation in the detail, such as adhesion around the graft, hardness, calcification or stone formation, stricture of ureter lumen, 4. Smooth muscle cell special marker, such as smoothlin should be assessed to evaluate the muscle layer within the vein graft, alpha SM actin is not Smooth muscle cell special marker as it also displays in myo-fibreblasts or fibroblasts.

*Answer to the reviewer:* We changed the gross observation accordingly to the reviewer’s request in the results section (page 6).

Changed passage:

During the explantation of the urological tract a mild scar formation was evident in the retroperitoneal space of all animals. No calcifications and no stone formation were detected. There was no significant difference in scar formation between the seeded and unseeded animals.

*Answer to the reviewer:* Regarding the use of smooth muscle alpha actin: We have specifically chosen smooth muscle actin, because it is also expressed in myofibroblasts, but not in fibroblasts. We wanted to avoid the opposite discussion that we are missing contractile cells (like myofibroblasts) in the veins with a highly specific marker like smoothlin. We also demonstrate that fibroblasts are not expressing smooth muscle alpha actin with a negative staining of the transplanted veins. For the positive staining for smooth muscle alpha actin of native ureters: I think everybody agrees that this is a smooth muscle layer.

5. Serum creatinine and urea tests are not unnecessary as one is for ureter graft and one is normal.

*Answer to the reviewer:* We agree with that, and changed this section accordingly. (page 7).

Changed passage:
The lack of an increased serum creatinine or urea in the hydronephrotic animals is explainable with the healthy contralateral kidney.

We hope that our answers to the reviewers are adequate. We would highly appreciate to hear back from you soon.

Best regards,

Georg Bartsch, M.D.

*Associate Professor in Urology,*
*Vice-Chairman*
*Department of Urology and Pediatric Urology*
*Goethe University*
*Frankfurt, Germany*