Author's response to reviews

Title: Immunohistochemical study on the expression of von Willebrand factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation

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Author's response to reviews: see over
Immunohistochemical study on the expression of von-Willebrand-Factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation

2nd REVISION

Cover letter

We have noticed the substantial improvement of our paper after the first review and are thankful for the reviewer’s time and help to improve the quality of our manuscript furthermore. We addressed all comments as outlined below. Text boxes have been added to the annotated manuscript indicating reviewer (R1; R2) with comment number (C1-4). Also revised sections of the manuscript have been changed using the track changes mode in MS word to facilitate review of the revision.

Reply to reviewer # 1

1. Concern of the reviewer:

   Title. This is a study comparing onlay bone grafts alone with onlay bone grafts in combination with DBBM and GTR. Among other things, the study looks at ingrowth of vasculature and connective tissue. It is not primarily a study of revascularization in onlay autogenous bone grafts. The title should reflect the true nature of the study.

   Our response:
   The title was changed accordingly.

   Revised text:
   Page 1 Immunohistochemical study on the expression of von Willebrand factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation

2. Concern of the reviewer:

   Abstract. The abstract needs to be rewritten to adequately discuss the experimental groups and findings. Only Experiment 2 is discussed in the abstract.

   Our response:
   The abstract was changed accordingly.

   Revised text:
   Page 2 Methods
Immunohistochemical study on the expression of von-Willebrand-Factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation

In a sheep model autogenous bone grafts were harvested from the iliac crest. A combination of a resorbable collagen membrane (CM) and deproteinized bovine bone material (DBBM) was used to modify the bone graft (experiment 2). This was compared with a simple onlay bone graft (control group, experiment 1). The amount of vessels in bone and connective tissue (CT), and the amount of CT were analyzed. The expression of von Willebrand factor (vWF) was compared between the two experimental groups using immunohistochemical analysis.

3. Concern of the reviewer:

The authors state they did not study onlay grafts with guided bone regeneration alone (the graft covered by a collagen membrane) because this was “described by Adeyemo et al. from our study group previously in the same experimental setting.” This work is cited in the manuscript, but the close association with the current study is not discussed. The current study is actually an extension of the earlier work, and this should be made quite clear.

Our response:

All changes were incorporated as requested.

Revised text:

...Previous studies also showed the superiority of a combination of DBBM and GBR with the use of collagen membrane (CM). This current study adopts the results of Adeyemo et al. and attempts to put an emphasis on the revascularization procedures within the graft in the same experimental setting [3, 4, 21]. DBBMs in general, are considered to be biocompatible and osteoconductive; however, evidence about their biodegradability remains inconclusive [22, 23].

4. Concern of the reviewer:

Needs some language corrections before being published

Our response:

The document was closely reviewed by a professional editing service for English scientific publications. All
Reply to Reviewer # 3

1. **Concern of the reviewer:**

   *The analysis of a mean vessel density as well as a morphological evidence of tubular vascular structures is not performed. When it comes to evaluating the (re-) vascularization of any tissue, the presence of vWF alone is no sufficient prove at all. Next to endothelial cells, vWF can also be detected in thrombocytes or megakaryocytes.*

   **Our response:**

   The use of vWF as a marker of the endothelium is one weakness of the current study. The fact that more specific stainings for markers such as CD31, CD34, and Fli-1 should be done in order to support the findings is mentioned on page 15. The fact that vWF can also be found in megakaryocytes and thrombocytes is added to the paper.

   **Revised text:**

   Page 15

   In the current study, vWF as a marker protein for the immunohistochemical study of angiogenesis. VWF (factor VIII-related antigen), synthesized by endothelial cells and megakaryocytes is a glycoprotein, that mediates platelet adhesion and stabilizes factor VIII at sites of vascular injury [38]. Moreover vWF can also be detected in thrombocytes. Although it is commonly used as an immunohistochemical marker for endothelial cells, stainings for other specific endothelial markers such as CD31, CD34, and Fli-1 should be performed in future studies to verify the current results [39]. Nevertheless, the increased expression in the ROI “recipient site” emphasizes the pattern of revascularization from the host site to the bone graft.

2. **Concern of the reviewer:**

   *This manuscript focuses on vascularization, however vessel structures are not attested by one single experiment in this setting.*

   **Our response:**

   Vessels in connective tissue and bone were graded in Higher Power Fields in histological analysis. However, other markers such as VEGF, VEGFR-1 and VEGFR-2 were analyzed in this experimental setting using
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immunohistochemistry and published previously as cited in the current paper. Given the mentioned weaknesses, the authors decided to put an emphasis on the immunohistochemical detection of vWF in this study, rather than vascularization in general and changed the title accordingly (See comment R3 C3).

Revised text: not applicable

3. **Concern of the reviewer:**

The authors should include a thorough IHC detection of vascular structures followed by a systematic analysis of mean vessel density to seriously talk about vascularization. Otherwise the title ‘Immunohistochemical detection of vWF after onlay autogenous iliac grafts for lateral alveolar ridge augmentation’ might be more suitable.

**Our response:**

The title was changed accordingly.

Revised text:

Page 1

Immunohistochemical study on the expression of von Willebrand factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation