Author’s response to reviews

Title: Vertical bone regeneration using rhBMP-2 and VEGF

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Version: 1 Date: 08 May 2017

Author’s response to reviews:

Point-by-point reply to Reviewer Comments

First of all, we would like to thank all three reviewers for their time to read our manuscript carefully and for providing us with supportive and very helpful comments. We fully agreed to all points regarding the issues raised by the first two reviewers and corrected the paper accordingly. The main amendments are highlighted in red.

Reviewer: 1

1. In general it has to mentioned, that the use of rh-BMP-2 and VEGF is nothing completely new and it should be questioned if the intended outcome of the animal experiment and the outcome presented here justifies suffer and death of 108 animals. The used amounts of cytokines are quite high and (unfortunately) the selected material, ICBM, shows a quit good bone regeneration itself. Nevertheless, carrier systems have to be investigated, but data about release / stability of the cytokines in vitro are missing and not cited, as far as i recognized.

We agree with Reviewer 1 that any animal study should contain as few animals as possible. Therefore, we chose the mandible and the tibia as implantation sites. Doing so, 108 dental implants were inserted in a total of 12 animals. Animal keeping, their treatment and the experiments were done under veterinary care and in accordance with current animal welfare directives.
The release kinetics of BMP-2 has been published by our group several years ago (for instance see Kübler et al., Effect of different factors on the bone forming properties of recombinant BMPs, Mund-, Kiefer- und Gesichtschirurgie, 2000, Sep;4 Suppl 2:S465-9., or Kübler et al., Inductive properties of recombinant human BMP-2 produced in a bacterial expression system, Int J Oral Maxillofacial Surg., 1998 Aug;27(4):305-9.)

Experiments dealing with the release kinetics of VEGF have also been performed in our lab, the results are about to be published and available for the reviewers on request on the basis of trust.

2. The Numbers given in the manuscript have 2 decimal places, representing an accuracy, which is for me incomprehensible, not necessary for discussion and questionable with respect to the measurement method. In addition, the standard deviations are quite high, wherefore the second decimal place has no meaning at all.

We agree with this comment and altered the manuscript accordingly.

3. Page 11, Line 10: "Significant difference was calculated" Please reword this part. The are calculations necessary to judge on a significance of the difference, but the difference itself is not calculated.

We corrected the sentence to „Highly significant differences were detected“.

4. Page 11, Line 14-17: This is a part I would like to know more about (data?) since it seems advantageous for planning animal experiments. Those observations should be emphasized to reduce redundant and unnecessary animal experiments.

We gave more detailed information about the implantation sites mandible and tibia. P. 11, Line 23- P.12, Line 3: „With the intention of using as few animals as possible, implants were inserted into the tibia and into the lower jaw. Although bone recovery differs and the exposure to bacteria in the oral cavity is missing, the tibia equals the lower jaw best in terms of bone size and volume. Furthermore, oral hygiene cannot be managed in mini-pigs, resulting in an increased loss of implants. Overall, implants inserted into the tibia showed higher values than implants inserted into the lower jaw but in order to get a reliable statistical outcome results could not be analysed separately”. Furthermore, we now provided the data in a new table for better illustration (table 2).
5. I would suggest to use bar charts insted of line charts, to incluclude standard deviations. Groups of Ctr, ICBM, ICBM+..., on the x-axis and the weeks as legend in different colors would be more instructive.

We followed your instructions exactly and altered the diagrams according to your suggestions.

6. Page 15, Line 11: "et al.: and others" can be deleted.

We followed your instructions and altered the text accordingly.

7. Figure 1 and 2: Please insert some arrows etc. to indicate regions of interest and explain them in the figure caption. Some histological relevant parts, tissues, cells might be helpful to interprete the images.

We inserted the arrows and explained them in the figure captions.

Reviewer #2:

1. Materials and methods

Page 7, line 21: Maybe I missed the information but I would suggest to state, how many implants were inserted in the tibia.

We now provide this information on page 7, line 21: „After 3 months 108 dental implants were inserted into the mandible (72 implants) and the tibia (36 implants) of the 12 mini pigs”.

2. Page 7: line 21 - page 8 line 5: The authors should state how wound closure has to be performed. Was a mucoperiosteal coverage achieved?

Yes, we now inserted this information on page 8, line 2-4: The periosteum was incised allowing the mucoperiosteal flap to tensionless cover the implanted area. Saliva-proof wound closure was performed by interrupted sutures using Vicryl 2.0.

3. Page 9, line 3: For a better understanding please use quadrat or square instead of cube as it is mentioned as two-dimensional (2 x 2 mm).
We followed your instructions and used square mm instead of „2x2 mm“. For sure, this is more clear that way.

4. Page 11, line 14-17: I am not sure whether it is helpful to summarize the values of the implants inserted in the tibia and the mandible. I understand the purpose of the authors to keep the number of animals as low as possible but let me address three issues:

1. The embryology of both areas is different. Whereas the mandible is mainly developed by desmoid osteogenesis the tibia is developed by chondral osteogenesis.

2. In the oral cavity there is a bacterial flora which may influence the healing of the implants. In the tibia, there should not be any bacterial flora.

3. As the teeth have been extracted 3 months prior to implant insertion there still might be some bone remodeling following the tooth extraction.

Therefore, in my opinion it would be more informative to divide the results by the different sites.

We followed your instructions and inserted a table providing the results divided by the different implantation sites (table 2).