Author's response to reviews

Title: The role of barrier membranes for guided bone regeneration and restoration of large bone defects: Current experimental and clinical evidence.

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Version: 3 Date: 9 April 2012

Author's response to reviews: see over
To: The Editorial Board of BMC Medicine

Re: Revised Manuscript submission (MS: 1049599959632861)

Dear Editors,

Please find submitted the revised manuscript entitled: "The role of barrier membranes for guided bone regeneration and restoration of large bone defects: Current experimental and clinical evidence." for consideration and publication in the BMC Medicine. We would like to thank you and the reviewers for your important recommendations for improving our manuscript. We have taken into consideration all the comments made and have replied point by point accordingly in the manuscript. Below is our detailed reply to each comment.

We would be most grateful if the article could be reconsidered for further review and potential publication. The authors of this manuscript declare that the article is original, that it is not under consideration by another journal, and that it has not been previously published. All authors have read and agreed to its content.

We are looking forward to hearing from you.

Sincerely

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Reply to reviewer’ comments

Reviewer: Robert Guldberg

Major Revisions

1. This review article summarizes work on membranes used for enhancing repair of large bone defects for craniofacial and orthopaedic applications. As noted, this concept has traditionally been applied for craniofacial applications but there is growing use for long bone defects and other orthopaedic applications. The repair of bone for these two broad categories of bone defects is typically very different in terms of the pathway of bone formation and the soft tissue boundary conditions. The review article does not consider that the "ideal" membrane may be different for different applications and in fact there is evidence for example that communication with the surrounding soft tissues is beneficial for long bone defect repair.

Reply to comment 1: We would like to thank you for your comment. In the initial manuscript, in the Discussion, in the section: - Long bone vs. maxillo-facial bone defects, we briefly mentioned the differences of the “local environment” and bone formation between long bones and the mandible. Based on your comments, we changed this section accordingly:

[According to the preliminary clinical reports, the time period for complete regeneration of bone in the mandible is 3 months, whereas long bones require more than two times the same period (7 months) [16,55]. This is most likely to be attributed to the greater vascularity of the mandible and the surrounding soft tissues as well as to the different mechanical environment and less stress-shielding of the fixation method used. Furthermore, it may also be explained by the different pathways of bone formation during the regeneration process due to the different embryological origin of the mandible (intramembranous ossification) compared to long bones (endochondral ossification) [161]. Considering these differences, the "ideal" barrier-membrane may be different for maxillofacial and orthopaedic applications. For example in case of long bone defects, the "ideal" membrane may require improved mechanical properties, prolonged degradation period in case of an absorbable membrane, and}
even different membrane porosity to allow vascular ingrowth from the surrounding soft tissues to optimize bone formation within the defect.]

2. The review article misses several key papers using degradable nanofiber mesh membranes recently published by the Guldberg and Hutmacher groups in Biomaterials, Bone, and PNAS.

Reply to comment 2: Thank you for your comment. As suggested, we included in the manuscript several key papers recently published by the Guldberg and Hutmacher groups using these novel nanofiber mesh membranes. These have been added in the relevant sections of the manuscript (see below). The reference list has also been corrected accordingly.

[Membranes and Growth factor release: ... Research is ongoing to develop novel membranes and scaffolds with improved growth factor delivery systems to accelerate bone regeneration of critically-sized segmental bone defects with promising preliminary results [95]. Moreover, with a controlled spatiotemporal delivery of growth factors, adequate local protein concentrations would be improved and maintained, avoiding the currently used supraphysiologic doses and the concomitant adverse effects [96].

Novel membranes and preliminary preclinical evidence: ...Novel membranes like polyethersulfone (PES) electrospun nanofibrous membranes have also been fabricated and loaded with cells to mimic natural bone. Such membranes have great potential for GBR, because of their three-dimensional structure and osteogenic bioactivity [107]. Another novel biomimetic tubular calcium phosphate (CaP)-coated nanofiber mesh combined with platelet rich plasma-mediated delivery of BMP-7 has also been recently evaluated for the regeneration of critical sized diaphyseal segmental bone defects in a small animal model with promising results [108].

Additional enhancement of bone regeneration with barrier membranes: ... Finally, in the future, improved barrier-membranes can be used as part of the bone-tissue engineering approach combined with osteoprogenitor cells and/or osteopromotive factors or even gene therapy, aiming to produce improved composite grafts [1]. Preliminary research is promising. For example, a novel three-dimensional
porous polymer Poly(ε-caprolactone) (PCL) scaffold coated with adeno-associated virus encoding BMP2 using both ex vivo or in vivo gene therapy, led to increased bone ingrowth with increased mechanical properties in a rat femoral defect model. [123].]

3. There is a need for further testing of these concepts in large animal long bone defect models.

Reply to comment 3: The need for more animals studies using large animal long bone defects models has been emphasized more in the manuscript by adding the following comments in the Abstract, Discussion and Conclusion.

[Abstract: … Reproducible results and long-term observations with barrier-membranes in animal studies and particularly in large animal models are required as well as well-designed clinical studies to evaluate their safety, efficacy and cost-effectiveness.

Discussion: … Even though there is extensive research on barrier-membranes in animals, human studies are still few. Therefore the most reliable current evidence originates mainly from studies in animals of higher phylogenetic scale which are still limited in number.

Conclusion: … Finally, reproducible results and long-term observations with certified barrier-membranes in animal models are required, and especially in large animal long bone defects models, as well as well-designed clinical studies to evaluate their safety, efficacy and cost-effectiveness.]

4. In addition to pore architecture, membrane surface microtopography is a potential important factor for optimization of membrane designs.

Reply to comment 4: We added the following sentence to the text in the section: “Additional enhancement of bone regeneration with barrier membranes”
[… Moreover, methods to optimise surface microtopography of the membranes are also been investigated to enhance bone formation at the cellular and molecular level [122].

Minor Revisions

1. There are several typos and spelling errors throughout the manuscript.

Thank you for your comment. Spelling and typographical errors have been corrected in the manuscript and tables.

2. In the abstract, please note that this is a review article not a study.

This has been corrected accordingly.

Reviewer: Dietmar Werner Hutmacher


Reply to comments: We would like to thank you for your comments. We acknowledge that our review does not give the scientific community new insights, but our aim was to perform a comprehensive overview of barrier membranes used for bone regeneration, summarise and present the current evidence from experimental and clinical studies, and focus on the specific requirements of such membranes for large bone defects and especially for orthopaedic applications. Regarding the several
reviews of high quality have been very recently published: the paper by Gentile et al. (2011) is an overview of different various non-resorbable and resorbable commercially available barrier membranes used specifically for guided bone regeneration in damaged alveolar sites before performing implants and fitting other dental appliances. Regarding the review paper by Calori et al (2011), this review focuses mainly on the different bone-graft substitutes used efficacy for the treatment of large bone defects and their efficacy in traumatology and orthopaedic surgery. However, our review focuses on the barrier membranes used for restoration of large bone defects in particular, including absorbable and non-absorbable membranes, their different types and characteristics, the current evidence from animal and clinical studies and future research on this field.

**Reviewer: A Masquelet**

One step procedure seems to be the main argument of the authors; nonetheless, in cases of defect resulting from infection or tumor excision we think it’s preferable to use a two steps procedure. Foreign body induced membrane has been conceived for this aim; Studies have showed that foreign body reaction is the key to understand formation of growth factors and angiogenesis. Thus a two stage procedure cannot be considered as a drawback. From this point of view, bioabsorbable synthetic membranes are worthy of interest, since they are likely to induce a foreign body reaction and to produce growth factors. On the other hand, the role of pore size is emphasized to obtain the penetration of vascular connective tissue through the membrane. The tissue infiltrating through the pores is said to differentiate into bone by direct or appositional bone formation. But what could be the precise process: biological process starting from the extremities of the bone defect or other. As the authors say «bioabsorbable membranes can also be used in combination with bone graft or bone substitutes and growth factors » The conclusion of the authors is relatively deceiving when they say that « the role of the membrane could be only a part of bone tissue engineering! One may regret that the authors have not given enough importance to the foreign body induced membrane which has biological properties favoring bone regeneration and which constitutes an excellent model of biological chamber for testing various combinations of osteoconductive and
osteoinductive materials. In summary, in the state of the art, it is difficult to conceive a bone regeneration in an important defect only by the means of a barrier membrane.

Reply to comments: Thank you for your comments. Please find below our reply to each comment.

Regarding the comment: However can we expect a spontaneous bone regeneration in a defect without osteoconductive material and osteoinductive substance? As the authors say « bone formation occurs only to the marginal stable zone with a central zone of disorganized loose connective tissue.»

Reply: In the section of “History and basic concept of guided bone regeneration”, we mention that in large defects and when additional bone grafting is not used, bone formation was found to occur only to the marginal stable zone with a central zone of disorganised loose connective tissue. Spontaneous bone regeneration, especially in cases of large bone defects, cannot be expected without osteoconductive material and osteoinductive substance, which is why we emphasize the importance of the additional use of bone graft material. We added the following sentence to the text: [Therefore, additional use of bone-graft materials is required in these cases, with the graft acting as a scaffold for osteoconduction and as a source of osteogenic and osteoinductive substances for lamellar bone formation [32].]

Regarding the comment: One step procedure seems to be the main argument of the authors; nonetheless, in cases of defect resulting from infection or tumor excision we think it’s preferable to use a two steps procedure. Foreign body induced membrane has been conceived for this aim; Studies have showed that foreign body reaction is the key to understand formation of growth factors and angiogenesis. Thus a two stage procedure cannot be considered as a drawback. From this point of view, bioabsorbable synthetic membranes are worthy of interest, since they are likely to induce a foreign body reaction and to produce growth factors.

Reply: We agree that, currently, the one step procedure for bone regeneration using barrier-membranes has known limitations for applications in large bone defects especially in cases of infection or tumor excision. We acknowledge the clinical applications of the induced membrane method for the restoration of large bone defects, and we rephrased the relevant paragraph in the Introduction as below:
Furthermore, the concept of an induced-membrane represents another strategy for bone regeneration and particularly in cases of large bone defects secondary to trauma, infection or tumour excision. This method involves a two-stage procedure, where a “biological” membrane is induced as a foreign body response after application of a cement spacer at the first stage, acting as a “chamber” for the insertion of autologous bone-graft at the second stage [12-14]. It has been shown that this induced membrane possesses osteoinductive, osteogenic and angiogenic properties, and several clinical studies have demonstrated satisfactory results [12,15].

Regarding the comment: On the other hand, the role of pore size is emphasized to obtain the penetration of vascular connective tissue through the membrane. The tissue infiltrating through the pores is said to differentiate into bone by direct or appositional bone formation. But what could be the precise process: biological process starting from the extremities of the bone defect or other. As the authors say «bioabsorbable membranes can also be used in combination with bone graft or bone substitutes and growth factors »

Reply: In the section: “Other clinical applications of bioabsorbable membranes”, we mention that: “...Bioabsorbable membranes can also be used in combination with bone graft or bone substitutes and growth factors to augment implants’ osseointegration in areas with bone defects [89].” This statement is referred to the potential clinical application of the membranes to improve the process of osseointegration of implants.

Regarding the comment: The conclusion of the authors is relatively deceiving when they say that « the role of the membrane could be only a part of bone tissue engineering! One may regret that the authors have not given enough importance to the foreign body induced membrane which has biological properties favoring bone regeneration and which constitutes an excellent model of biological chamber for testing various combinations of osteoconductive and osteoinductive materials. In summary, in the state of the art, it is difficult to conceive a bone regeneration in an important defect only by the means of a barrier membrane.

Reply: We agree that, currently, successful bone regeneration in an important defect cannot be achieved only by the means of a barrier membrane. We do acknowledge in
the manuscript the concept of the induced membrane as an important method for bone regeneration of large bone defects, but the goal of our review was to present a summary of the existing clinical and experimental data on the field of bone regeneration using barrier-membranes and to discuss the prons and cons of this method and specific considerations regarding its limitations and its limited indications for clinical use. Hence, we comment on the need for further research to establish the “ideal” barrier-membrane and delineate the need for additional bone grafting materials, aiming to “mimic” or even accelerate the normal process of bone formation.