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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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 (please find each reply in bold within the text). 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’s 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) [33,57]. 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) [135]. 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 have 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 and Tables (see below). The reference list has also been corrected accordingly.


In the Introduction, in the subsection “The role of mechanical stability”:

… New vascular network formation, which is a prerequisite for bone formation, is also highly sensitive to mechanical conditions with delayed mechanical loading significantly enhancing bone formation and stimulating vascular remodelling by increasing the number of large vessels and decreasing the number of small vessels [90].


In the Discussion, in the subsection “Biological augmentation of GBR with growth factors”:

[…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 [150]. Moreover, with a controlled spatiotemporal delivery of growth factors, adequate local protein concentrations can be improved and maintained for optimal regenerative efficacy, avoiding the currently used supraphysiologic doses and the concomitant adverse effects [151].


Also, in the subsection “- Other strategies to improve bone regeneration”:

[...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 [158].


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: In the Introduction, we added the subsection: - The role of porosity and topography of the barrier-membranes with the relevant text and reference.

[…In addition to the porosity, the tri-dimensional topography of the membrane with interconnecting pores and channels is also important, as it can alter the cell occlusion properties and the biologic response of different cell types to the membrane [83].


We also added the following sentence to the text in the Discussion:

[…. Moreover, methods to optimise surface microtopography of the membranes are also been investigated to enhance bone formation at the cellular and molecular level [157].

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 «bioabsobable 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: We made substantial revisions to the manuscript according to the Editor’s comments and for this comment in the Introduction 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 (in bold) as below:
[…However, in large defects, bone formation occurs only to the marginal stable zone with a central zone of disorganised loose connective tissue; and 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 [23].]

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 [9-11]. It has been shown that this induced membrane possesses osteoinductive, osteogenic and angiogenic properties, and several clinical studies have demonstrated satisfactory results [9,12].

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: This has been rewritten (briefly) in the section: “The role of mechanical stability”

[It is known that micromovements between bone and any implanted material prevent bone formation, resulting in the development of fibrous tissue [87,88]. Adequate stability and minimal stress are required to allow the early tissue that infiltrates through the pores to differentiate into bone by direct or appositional bone formation [81]. Bone formation can occur within porous materials even with limited initial movement; provided the site is highly vascular and local inflammatory reaction is minimal [89].

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.
Editorial comments:

1. Abstract: I have changed the wording here slightly to clarify that this is a review rather than a study.

   Thank you. This change has been made in the revised manuscript.

2. Introduction: Although I am not an expert in this field, it seems that much of the content from the sections ?History and basic concept of guided bone regeneration? up to and including ?Current evidence for in vitro studies?(pages 1-11) have been previously covered in other recent reviews, which is one of the concerns that reviewer 2 had. Therefore, I would suggest that you draft a new introduction which briefly includes and outlines the content of these sections as background rather than in depth information, and refers the reader to these other reviews or primary literature.

   I realise this is a rather large section to condense and co-opt into your introduction, so it may also be appropriate to put some of the details into tables to which the interested reader could refer, and to which you could briefly outline in your text, while highlighting the most important issues.

   The topics I thought would be appropriate to detail within tables were: ?Types of barrier membranes and their basic characteristics?, (which includes the subsections ?Non-resorbable membranes?, ?Bioresorbable membranes?, ?Collagen membranes?, ?Chitosan membranes?and ?Synthetic membranes?) and ?The role of mechanical stability when using membranes for bone regeneration? (which includes ?Fixation of membrane?, ?Effect of type of fixation?, and ?Additional cortical perforation?). Removal of much of discussion in the text that overlaps with previously published reviews will then allow you to expand on some points that the reviewers have highlighted. I have also made notes within specific sections in the marked up Word document regarding the points that I feel should be expanded/further discussed.

   Reply: Substantial changes have been made in the manuscript based on your recommendations. All your comments have been addressed in the revised version of our paper and a Table (Table 1) has been added as you suggested. The mechanical stability was left in the main text as a subsection in the Introduction as it was not possible to include such information in a table, but the content was significantly condensed.
3. Discussion that the two-stage procedure cannot be considered a drawback because studies have shown that foreign body induced membrane stimulates angiogenesis and growth factors (noted by reviewer 3). Please briefly discuss this point citing relevant research.

Reply: Thank you. Please see our reply to reviewer 3 regarding the comment on the foreign body induced membranes. The following changes were made in the relevant paragraph in the Introduction.

4. Bioresorbable membranes: Reviewer 1 notes that this review misses several key papers using degradable nanofiber mesh membranes recently published by the Guldberg and Hutmacher groups in Biomaterials, Bone, and PNAS, so please add this to the citations to which the interested reader could refer. Reviewer 3 notes that bioabsorbable synthetic membranes are worthy of interest since they are likely to induce a foreign body reaction and to produce growth factors, so please briefly expand upon this point.

Reply: Please see our reply to Reviewer 1 comment. We added a number of papers in the text and the reference list.

5. Collagen membranes/Chitosan membranes: I assume that collagen and chitosan membranes are not currently available for clinical use? Please do clarify this. If, however, they are being used clinically, please detail the prevalence of use, which may be put in a table if necessary.

Reply: Thank you for your comment: The following text has been added in the Introduction: [A summary of the main characteristics, advantages and disadvantages of the different bioresorbable membranes is presented in Table 1 [13,21,31,38,40-58]. Currently, mainly PLLA membranes are available for clinical use in orthopaedic surgery; whereas PLLA, collagen and ePTFE membranes are used for GBR in maxillofacial, dental and neuro-surgery.]

6. Role of pore size in barrier membranes: Reviewer 1 notes that in addition to pore architecture, membrane surface microtopography is a potential important factor for optimization of membrane designs, so please could you discuss this. Soft tissue ingrowth: In order to be a little more accessible for non-specialists, please detail what soft tissue ingrowth is. Please also discuss the clinical
implications of this. Bone absorption: Please discuss the clinical significance of the findings you highlight. Effect of types of fixation: This is informative, but needs to include a little more insight. Please discuss within the text your recommendations for the type of fixation, specifying examples of the type of fixation required for each situation. Additional cortical perforation: Please briefly explain what cortical perforation is. Please also round this off by providing your own insight and opinion into the findings. Current evidence for in vitro studies: This could be integrated into the areas you discuss previously (collagen membranes, synthetic membranes).

Membranes and growth factor release: In this section, you discuss here results from various studies that are controversial in nature. What would strengthen this section would be to have your own opinions as to the controversy in these studies. Please add a couple of sentences to the end of this section on what you think this research means.

Reply: All the above comments have been addressed in the text and significant changes were made in the manuscript. We integrated the in vitro evidence into the text and table 1. We also briefly explained the concept of cortical perforation (in the Discussion, as below):

… The evidence on the efficacy for cortical perforation (decortication) during GBR procedures in an effort to enhance bone formation remains controversial [142]. Studies have shown that cortical perforations increased the blood supply, facilitate angiogenesis, and allow access for progenitor cells from the bone marrow into the “chamber” [142]; whereas other studies showed that bone formation occurred from a non-injured cortical bone surface and that perforations were not required as they did not increase bone formation [59,81]. However, since there are no relevant human clinical studies and the relevant animal studies refer to mandibular defects, where local vascularity is superior to long bones, recommendations for additional bone decortication cannot be made for orthopaedic GBR applications [142].

We also commented on the existing controversy regarding the studies evaluating the use of growth factors in bone regeneration. (In the Discussion, as below.)

[Controversial evidence may be secondary to insufficiency in maintaining therapeutic concentrations of growth factors within bone defects due to rapid clearance and use of different delivery methods with supraphysiological non-standarised doses to obtain
therapeutic efficacy [147]. Furthermore, current research usually evaluates one or combination of two growth factors, which does not reflect the complex physiological process of bone formation.]

Regarding the fixation type, in the Introduction in the section of the role of the mechanical stability, we added the following sentence:

*It has been demonstrated in vivo that there is more rapid and more organised new bone formation in rigidly fixed defects with plate osteosynthesis, covered with a resorbable collagen membrane, compared to non-rigidly fixed defects [94].*

4. Novel membranes and preliminary preclinical evidence: This was a section that I found to be particularly interesting. Before discussing the specific novel membranes, please briefly discuss here why such novel membranes have been developed.

Reply: The following text has been added in the Introduction:

[…Although a number of barrier-membranes are already being used in the clinical practice, novel membranes have been developed in an effort to overcome the limitations of the currently used membranes. Such novel membranes include alginate membranes, new degradable co-polymers, hybrid or nanofibrous membranes, as well as amniotic membranes. They are summarised in Table 1 [60-75]. Ongoing research is evaluating these novel membranes, aiming to establish an “ideal” membrane for bone regeneration with optimised characteristics in terms of biocompatibility, space-making, tissue integration and clinical manageability for maximum clinical efficacy and safety.]

5. Literature Review and Discussion: Here, you describe your literature review, and briefly discuss in the animal studies and clinical studies the articles you assessed, before going into a discussion. I wasn’t clear whether the discussion was a discussion on the literature review. If this is the case, please do clarify this, ignoring my points 23 and 24. I also suggest you start off this section by giving a brief rationale as to why you performed this literature review.

Reply: In the section of “Literature Review” we added the following comment:
As the research on the field of bone regeneration is ongoing and the evidence is expanding, we aimed to summarise the current experimental and clinical research on the use of barrier-membranes for restoration of bone defects and focus on maxillofacial and orthopaedic applications.

In the Results in the subsection of Animal and Clinical studies, we briefly discussed the main findings from the studies presented in the tables as per your suggestion.

6. Please could you also discuss the implications of the difference in bone quality in terms of the trials you highlight? I would assume that this would mean we should treat the results with caution?

Reply: This has been commented in the Discussion:

…Findings from the experimental setting indicate that GBR follows the same course of steps regardless of the animal. Bone quality though is highly dependent on the species (evolution hierarchy), bone healing potential (age, general nutritional status), the membrane used, local conditions (vascularity, embryological origin of bone) and load-sharing pattern of the fixation method; and therefore the results and the potential clinical use should be interpreted with caution [2,13,33,76,123,130].

7. Specific considerations for orthopedic surgery: Please add the relevant citation here.

Reply: The relevant papers were added in the text and the reference list.


8. List of abbreviations: There are numerous abbreviations here, so please add this section. If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors’ contributions.

Reply: A list of abbreviations has been added.