Author’s response to reviews

Title: Functional Tissue Engineering of Ligament Healing

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Author’s Replies to Reviewers’ Comments

--- We thank the reviewers for the detailed comments and critical review. We especially appreciate the positive comments which stated “This is an excellent review, well written, clear in its message, up to date references but still historic data enclosed.” We believe the comments are helpful and have allowed us to significantly improve our manuscript. The following is our point-by-point responses.

Reviewers' comments:

Reviewer #1:

General Comments:

The authors aimed to review the current development of functional tissue engineering techniques to promote of ligaments and tendons healing. However, the content was in fact, mainly on healing of ligaments, or more specially, MCL and ACL, which the authors are most familiar with. There is an in-balance in the discussion of tendons and ligaments. It is therefore advised to change the title and modify the content to make the article more focused.

--- Thank you for the comment. We have made changes to the title and content so that our review is now mainly focusing on the discussion of ligaments. Please see the highlighted areas.

Specific Comments:

Comment 1. In the abstract, the authors mentioned that “the healing responses are different in the ligaments and tendons after injury, the consequences and treatments are tissue- and site-specific”. Please elaborate how the healing responses and treatment are different …
--- Since it is an overall summarizing sentence in the abstract, we think it is better to elaborate on the point in the manuscript. However, we added a sentence to clarify that the healing of ligaments would be emphasized in the review.

Comment 2, 4 - 7, 10 - 15, 18 - 21, 23, 25 – 38, 40 - 46, 49, 50, 52-60.
--- These are good points. We have made corresponding changes that are highlighted in the revised manuscript.

Comment 3, 8, 9, 22, 24, 39, and 51. Please add the reference.
--- In these comments, references were preferred to be added to some general statements. However, some of the statements are in the Introduction (Comment 3, 8, and 9), for which we elaborated later in the text with appropriate references. The other statements (Comment 22, 24, 39, and 51) have following explanation statements with references. Therefore, we think it will appear redundant and confusing if we add in the references again to the general statements.

Comment 15. “Moreover, even though the morphological appearances of some ligaments and tendons are similar to each other, ….” Please add references and elaborate this point further about how they are different in terms of biochemistry and biomechanical properties.
--- We have added the references. There are quite a few differences in terms of biochemistry (collagen content, subtype ratios, GAG content, DNA content, etc.), and biomechanical properties (structural, mechanical and viscoelastic properties) between ligaments and tendons as well as different ligaments (e.g. MCL and ACL). However, the purpose of this part of review is to familiarize the readers with the basic knowledge and healing process of dense regular connective tissue so that we can lead the reader easily to the issues of ligament healing and the application of functional tissue engineering approaches. To elaborate on the differences between different ligaments and tendons seems aberrant from our original aims in this review.
Comment 16. “their environment would have profound effects on their healing capabilities.” Please add references and elaborate this point further how the environment affects the healing capabilities of ligaments as well as tendons.

Comment 17. The authors has described the healing of extra-articular ligament. What kind of model it is? How about healing in intra-articular ligament and tendons? Are they the same?

--- By saying ‘environment’, we focused on the healing of extra-articular ligament (MCL) and intra-articular ligament (ACL). The healing of MCL is classical and was discussed in Section II while the healing of ACL is unique and was discussed in section III. Their healing processes are different, which are closely related to their locations in different environment. We have made modifications to the sentences so that they are easier to understand. The references are with the detailed statements.

Comment 31. “For patients over 40 years of age that have proxial…this procedure has successful results [66].” I read the title of [66] and it was on skeletally-immature patients. However, you were talking about patients over 40 years of age. Please clarify.

--- We apologize on the negligence. The reference was not correct. We have put the correct reference in the revised manuscript.

Comment 35. The authors refer references [67-71] as earlier studies and others such as [74-81] as more recent studies. However, references such as [69] in fact is quite new while [81] is quite old. Please clarify.

--- We apologize for the confusion. By saying ‘earlier work’, we meant the previous studies. Those studies were given as examples to explain that growth factors, cytokines and stem cells were used as biological augmentations to improve ACL healing. The [81] was a reference in the part of using growth factors. There was not a chronicle description. We have modified the sentence to make it clearer.

Comment 47. “Further, when an MSC-seeded implant was delivered to a Achilles tendon..” should be “Further, when a MSC-seeded implant was delivered to an Achilles tendon…” Moreover, this session is about ACL healing, I would suggest
including references on ligament healing only.

Comment 48. “Similarly, an autologous MSC collagen graft could accelerate…patellar tendon in rabbit.” Similar comment as in #48.

--- Indeed this session is about ACL healing. However, we feel that it will be a good supplement to the background by introducing the previous studies of using MSCs in Achilles tendon and patellar tendon first. It will be helpful for readers to understand what we want to confer. Meanwhile, we have made the suggested changes in the sentence.

Comment 54. Future directions, “Research to enhance … both biological and biomechanical augmentation can be used to improve their outcome.” There is in fact, little coverage on the tendon healing and no discussion on biomechanical means to improve the outcome.

--- We have made modifications to the sentence in the revised manuscript. However, we prefer to keep the ‘biomechanical augmentation’ in the sentence because this statement is a general comment on the current developments of ligamentous tissue healing.

Comment 56. “With the reduction or elimination of the immunogenicity from the …..its usage will be more acceptable and wide spread.” Did you observe any sign of immuno-rejection in your studies or in previous studies using SIS?

--- In non-primate animal studies, the immuno-rejection reaction is not an issue because they do not have the anti-αGal antibodies. However, in human studies, especially in the study of rotator cuff tears, there are adverse reports regarding the xeno-material issues. The references have been added.

61. There is no scale bar for figure 1.

--- The image was derived from a previous study in which we used magnification instead of scale bar for the dimension. The purpose of this figure was to demonstrate the organized collagen fibers and spindle shaped fibroblasts, so we
enlarged a portion of the original picture. In the revised manuscript, we included the original image at 200 of magnification. The legend is also changed according.

62. Figure 2 legend, how do you know that the collagen fibers were newly formed?

--- The images in Figure 2 were also derived from a previous study. In the study, we create a large gap (6mm) injury in the MCL in rabbit. When we took the healing tissue from the midsubstance of the healing ligaments for TEM analysis, it was certain that no normal or other surrounding tissue was included (the healing tissue was easy to identify because of the large gap). Compared to the homogenous small collagen fibrils in the non-treated group, we found larger collagen fibrils in the SIS-treated group. The possibility that those large collagen fibrils were the remnant of SIS was excluded because the range of fibril diameters for the SIS itself was smaller than the newly found large fibrils after measuring with software (Simple PCI). In addition, the size of the large collagen fibrils increased with time as we found in our studies at later time points. Therefore, we think it is highly possible that the large collagen fibrils in the SIS-treated group were newly formed.

Reviewer #2:

Comment 1. Page 15, 1st paragraph, “…healing[99]..” should read “…healing[99].”

--- Thank you. The change has been made in the revised manuscript.

Comment 2. Non-viral gene transfer should be mentioned as it is well known that viral transfection has potential risk and efforts in this area have been done.

Comment 3. Biomechanical stimulation during FTE using bioreactors should also be addressed as it is quite important to attain functional tissue replacement and a lot of work has been done.

--- These are good points. For the current manuscript, we would like to focus on reviewing the functional tissue engineering approaches used in ACL healing.

Therefore, we prefer to include the suggested contents in our future publications.