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Professor Shui Qiu
Science Editor
World Journal of Stem Cells

Re Manuscript Number 23217: Endometrial Mesenchymal Stem Cells as a Cell Based Therapy for Pelvic Organ Prolapse, by SJ Emmerson and CE Gargett

Dear Professor Shui Qiu,

Thank you for your email dated 30th November confirming the completion of our manuscripts review. We thank the reviewers for their favourable comments and we are delighted that it has been recommended for publication following minor revisions. We wish to thank one reviewer for his/her helpful suggestions, which we have addressed in this revision. We believe the manuscript has been improved as a result.

Please note that we have added another figure as a result of the revision, a new Figure 5. Our previous figures (5-6) have been renumbered Figures 6-7, respectively.

Please find our point by point response to the reviewers' comments below, quoted exactly as presented.

Reviewer # 00742243

"none"

Reviewer: # 00505901

"The manuscript is well prepared with important academic merit. The reviewer recommend the acceptance."

Reviewer: # 02929620

Well written, interesting article that will contribute to the current literature on stem cells.

Review: # 00680693

"The present review is a detailed analysis of the possible cell sources for a tissue engineering application for the treatment of pelvic prolapse. In particular, the authors focus on endometrial mesenchymal stem cells, providing interesting analysis of characterization,

isolation as well as use in scaffolds. The review is sound and interesting and I enjoyed reading it.

I would suggest a small discussion on the possible limits of MSC isolation and use (alterations in diseased patients such as endometriotic patients)

Response: The reviewer makes an important point as conditions such as endometriosis are very common affecting 10% of young reproductive age women who are frequently infertile and therefore have not had the opportunity to develop POP. The majority of women undergoing pelvic organ prolapse surgery are postmenopausal and endometriosis subsides in menopause due to estrogen deficiency (it is an estrogen-dependent disorder). We have shown that short term estrogen replacement therapy regenerates human endometrium of postmenopausal women but this could reactivate endometriosis but only for a short term – it would be safe under the care of a gynaecologist. We have added the following on pages 12 line 3-14:

"Despite their great promise, eMSC and menstrual blood MSC have yet to be significantly explored as therapeutic agents of stem cells therapy. There are certain endometrial disorders where caution maybe required eg endometriosis. However this disorder affects young infertile women who will not have the opportunity to develop POP. Indeed, it will be important to ensure no underlying uterine or other pathology (eg any malignant tumor) in identifying suitable patients for cell harvesting to treat their POP. For example, should a woman have uterine cancer, it would not be possible to use her eMSC for cell-based therapies. Similarly, it would also be contraindicated to use another source of autologous MSC in case tumor cells have spread to organs such as bone. These important issues should be considered in developing the potential of eMSC as cell-based therapies."

General MSC problems in diabetic patients, or even possible risks of maldifferentiation could also be discussed.

Response: Although MSCs have been proposed as a possible treatment for diabetes, it is currently unclear whether diabetes itself causes any problems with MSCs. Furthermore no literature could be found that illuminated this problem. Indeed, most literature focussed on the use of MSCs for the treatment of diabetes. However we have added a small paragraph on the limitations of MSC as a cell-based therapy. We have added the following on page 9 lines 17-31:

"Although MSC show promise as cell-based therapies, more understanding of their mechanism of action and utilising their potential is needed. Early use of MSCs has not always met expectations, often producing inconsistent results [55]. This may be due to lesser refined methods of isolating and cultivating MSCs resulting in the administration of fibroblasts and myofibroblasts rather than undifferentiated MSC [56]. Until recently, production of significant numbers of MSCs posed a challenge, as the regenerative potential of MSCs declined during culture expansion [57,58], which is required due to the small numbers of perivascular MSCs present within tissues [59]. For tissue engineering applications and tissue repair following ischaemia (eg cardiac muscle), local rather than systemic delivery is desirable and will likely result in greater local concentration of MSC at the desired tissue site, even when the mechanism of action is paracrine [60]. A further consideration is allogeneic versus autologous. Seeding MSCs onto scaffolds, such as polyamide/gelatin (PA+G) for POP or poly-lactic-co-glycolic acid nano-fibers appears to produce better outcomes in preclinical studies [57, 61]"

1. *Regarding the generation and implantation of a cellularized autograft for POP, a scheme could be helpful for the reader to better understand the procedures involved.*

   Response: We thank the reviewer for this suggestion. We have now included a flow chart as a new Figure showing a schematic covering the acquisition of eMSCs, their purification, culture expansion in near GMP conditions through to large animal models and clinical trials for implanting them on mesh into women with POP for inclusion in the section **"Isolation and application of eMSC in POP vaginal repair"**. The new Figure 5 legend is detailed below.

"Figure 5: Isolation and application of eMSC in POP vaginal repair: A) simple office based endometrial biopsies can be used to obtain patients' tissues, which are dissociated, then **B)** eMSC selected using SUSD2 magnetic bead sorting, followed by **C)** culture expansion in A83-01/serum free medium in 5% O_2 to generate large numbers of undifferentiated SUSD2+ eMSC (90-95%) for **D)** seeding onto fabricated scaffolds which will create an **E)** eMSC/PA-G tissue engineering construct for implantation into **F)** a large animal preclinical model to assess their efficacy in vaginal repair of parous ewes with evidence of POP. "

Its **(Fig. 5)** insertion into the text was first placed on line 5 of page 12 and again on line 16 on page 17.

Additionally, we were unable to remove all of the arrows and annotations from the images in our review. Figure 1 features images that we have used with permission from a biotechnology company, but we did not have access to the unaltered original images.

We look forward to hearing the final outcome of our review article and eventually seeing it published on line.

Yours sincerely,

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