Author's response to reviews

Title: Edible Bird's Nest Extract As Chondro-protective agent for Human Chondrocytes Isolated from Osteoarthritic Knee

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Version: 4 Date: 25 October 2012

Author's response to reviews: see over
Dear Editor,

Re: Resubmit manuscript after revision
Manuscript ID: MS: 1973775077635404

Manuscript title: Edible Bird’s Nest Extract as Chondro-protective Agent for Human Chondrocytes Isolated from Osteoarthritic Knee.

New Title: Edible Bird’s Nest Extract as Chondro-protective Agent for Human Chondrocytes Isolated from Osteoarthritic Knee: In Vitro Study.

Thank you for your kind comments on the above manuscript. We have made the recommended changes according to the reviewers’ comments and resubmit this manuscript for your reconsideration.

Enclosed here is the outlining of the changes that have been made in the manuscript and the corrected version of manuscript.

Thank you and looking forward to hear from you soon.

Yours Sincerely,

Dr Chua Kien Hui (On behalf of Dr Lee Ting Hun)
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The amendments are made according to the reviewer’s comments. Amendments to the original manuscript are stated below for its specific location.

Respond to reviewers’ comments

Reviewer : 1

Specific Comments: The English language in the manuscript text requires a complete overhaul.
Amendment: We have rewritten the manuscript and corrected the language error.

1. Comment : the title is slightly misleading.
Answer: We have changed the title to justify the current study is an in vitro study. Chondro-protective means to promote wellbeing of the cultured chondrocytes by EBN extract.
Amendment: New Title
Edible Bird’s Nest Extract as Chondro-protective Agent for Human Chondrocytes Isolated from Osteoarthritic Knee: In Vitro Study.

2. Comment on Abstract: Please edit the abstract carefully for correct use of scientific English.
Amendment: We have edited the abstract using scientific English.

3. Comment on Introduction: The introduction (background) appears to be too long. This section should be more focused and concise.
Amendment: We have rewritten the introduction into a more concise form.

4a. Comment: this reviewer has major concerns about extraction of EBN using hot water. What is the scientific rationale for this? Although EBN extracts are prepared by adding hot water in Chinese traditional medicine, this does not mean that it is the most valid method of extraction. The authors should discuss why methanol, ethanol, chloroform and DMSO were not used. The use of hot water for the extraction process needs to be fully justified.
Answer: We have justified the reason of using hot water extraction in the Introduction.
Amendment in Introduction page 5.

4b. Comment: Also, the reasons for storage at 4 degrees centigrade should be clearly explained.
Answer: We have explained the reason of storage at 4 degrees centigrade in the introduction.
Amendment in Introduction – last paragraph of page 5 and first paragraph in page 6.

5. Comment: The authors have used chondrocytes from OA joints from 6 patients. This seems rather odd because the majority of investigators in this area of research use normal chondrocytes from HEALTHY and NORMAL JOINTS and subsequently stimulate them with pro-inflammatory cytokines to induce an OA-like environment in vitro. Why did the authors not use this standardized approach? OA is a very complex and heterogeneous disease and there are likely to be many differences between the chondrocytes isolated from OA joints of different patients. In terms of biological response and phenotype, each patient is likely to respond differently. There is no mention of the grade of OA in each of the samples used. How can the authors be confident that the cells isolated from 6 patients are homogeneous? This is a major criticism and needs to be addressed.
Answer: We used HACs isolated from OA cartilage in order to provide a more relevant data with appropriate cellular model, since the cells has expose to natural OA environment rather than using the induced normal articular chondrocytes with IL-1 in the culture.
Amendment at Methods: page 6.

AND

Answer: The homogeneous of the specimen was determined by the professional judgement of the orthopaedic surgeon. All patients had OA knee with lesion scored by International Cartilage Repair Society (ICRS) of grade 4. The cartilage is harvested from non-weight bearing area of intercondylar notch distal femur. Whereby there must be a minimum weight of 300mg remnant good cartilage in the area. In addition, the isolated HACs must expressed collagen type II mRNA at the ± 20% differences from min.
Amendment at Methods: page 6.
6. Comment: The MTT assay is not really a suitable assay because it is a proliferation assay rather than a viability assay. Why did the authors not use LIVE/DEAD assays or similar viability assays?

Answer: EBN supplementation increased HACs proliferation at low concentration until 1.00% (v/v). LIVE/DEAD assay or viability assay is frequently use for testing compound which mainly cause toxicity to the cells. Since EBN does increased HACs proliferation compared to the control group (without EBN supplementation), we still recommend MTT assay to determine the suitable dosage of EBN.

7. Comment: The discussion can be more focused and structured. The conclusion is clear and the authors have cited a reasonable number of references.

Amendment: We have made the Discussion more focused and structured accordingly. We also changed a few references that more relevant to the new Discussion.

8. Comment: Tables 1 and 2 are clear.

9. Comment: The figures require significant improvements. The quality and resolution of all the figures presented is poor. The figures are not sharp enough and the files appear to be of very low resolution.

Amendment: We have improved the quality of all the figures and resubmit it.

10. The use of color is not scientifically justified in the graphs. There is no scientific reason for the control bars to be plotted in red.

Amendment: We have use the standard black color for the control in all figures.

Second Reviewer's report:
I query the import of a study with only 6 participants. I appreciate that this study is not a clinical trial, and that there may be considerable difficulties in having high numbers of participants consent to providing live cartilage samples.
Answer: We have used a standard statistical test to determine the number of specimen needed in this study. The test showed N=6 is sufficient. This test has been validated by a medical statistician from our institution.

Minor revisions

Bird's nest and birds' nest are used interchangeably throughout the manuscript. Please select either the singular or the plural form and use it consistently.

Amendment: We have used bird’s nest in the manuscript.

Write abbreviations in full at first use (see abstract). The abbreviation will suffice thereafter (eg: HMG, COX-2, SOX-9)

Amendment: We have made the correction in the abstract. HMG was a code given to the EBN extract from the institution, it isn’t an abbreviation.

The penultimate sentence on p. 5 should read "The structural rigidity of...".

Amendment: We have made the correction.

Discretionary revisions:

The writing will be cleaner if you delete unnecessary phrases such as "It is well known that...", and simply state the thing that is well known (see page 4).

Amendment: We have made the correction.

Editor’s comment: Patient consent

Amendment: We have clearly state in the Methods that, patients had given written consent for using their cartilage in the study; Page 6.