PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Adipose derived mesenchymal stem cell therapy in the treatment of isolated knee chondral lesions : design of a randomised controlled pilot study comparing arthroscopic microfracture versus arthroscopic microfracture combined with post operative mesenchymal stem cell injections.
AUTHORS	Freitag, Julien; Ford, Jon; Bates, Dan; Boyd, Richard; Hahne, Andrew; Wang, Yuanyuan; Cicuttini, Flavia; Huguenin, Leesa; Norsworthy, Cameron; Shah, Kiran

VERSION 1 - REVIEW

REVIEWER	William D. Murrell, MD, MS Humeira Badsha Medical Center, UAE Ft. Belvoir Community Hospital, USA Author is PI for trial involving administration of peripheral blood progenitor cells and hyaluronic acid adjuvant treatment to augment modified subchondral drilling for knee cartilage regeneration.
REVIEW RETURNED	14-Aug-2015

GENERAL COMMENTS	Overall, the protocol is a well-planned and organized trial with great ambitions to provide additional safety and efficacy data for use of mesenchymal stem cells to promote repair of knee articular cartilage. Hopefully, new data from this trial will further assist in the translation of cellular based therapies from clinical investigations into mainstream, and widespread treatments. The current authors have leveraged data from a previously published trial using peripheral blood progenitor cells with modified subchondral drilling and postoperative adjuvant hyaluronic acid or hyaluronic acid and peripheral blood progenitor cells injections to regenerate knee articular cartilage. What makes this trial unique is that culture expanded adipose derived stem cells are used instead of hyaluronic acid and peripheral blood progenitor cells obtained by apheresis. In the design of trial, using a single intervention or treatment is attractive because it will virtually eliminate any confounding variables due to multiple or competing treatments. This study will preliminarily answer the question: Do culture expanded adipose derived mesenchymal stem cells help to promote the repair/regeneration of knee articular cartilage in humans undergoing arthroscopic micro fracture? The protocol should be published with minor revisions. Abstract

	Would recommend changing line 18 to: Technically challenging, expensive and may not have significant improvement over microfracture alone. Would recommend expanding line 23 to include the source of cells: culture expanded…… Line 29 would recommend changing statement in parentheses to (microfracture only or microfracture alone) Line 30 would recommend changing statement in parentheses to (injected adjuvant culture expanded adipose derived stem cells administered post-operatively) Comment-Line 39 - The cartilage regeneration may be underwhelming at 12 months, however from a scientific follow-up perspective this is acceptable. Caution-however, if the regeneration is underwhelming in this time frame, the risk of demonstrating experimentally that the great expense of using adjuvant stem cells to improve regeneration is certainly a risk. Background Line 28-recommend including additional reference with reference 5: Failures, re-operations, and complications after autologous chondrocyte implantation--a systematic review. Harris JD, Siston RA, Brophy RH, Lattermann C, Carey JL, Flanigan DC. Osteoarthritis Cartilage. 2011 Jul;19(7):779-91. doi: 10.1016/j.joca.2011.02.010. Epub 2011 Feb 17. Review. PMID: 21333744 [PubMed - indexed for MEDLINE] Page 5/21, line 22 recommend adding additional paragraph: Proof of concept as far as cartilage repair or regeneration requires 3 main requisites. This requires demonstration of adequate production of proteoglycan, predominance of type II collagen, and cartilage structural reconstitution. Page 5/21, Line 29 delete: (containing bone marrow derived MSCs) as the marrow aspirates were not characterized in this study, and MSCs may or may not have been present. Page 5/21, line 30 insert: Enhanced tissue repair (with increased proteoglycan production histologically, following....) Page 5/21, line 38 delete microfracture—in the report it is clearly modified subchondral drilling, and Pridie method is referenced in the original article. Page 5/21, line 39 change 12 to 18 months. Only postoperative outcome that was recorded at 12 months was IKDC score. Biopsies and MRI were done at 18 months. Page 5/21, line 41, this sentence needs to be modified. Better to say that additional work is needed because of multiple treatments are confounding variables found in this study, i.e., HA and PBPCs. Would not because of the age difference between the groups. This statement is misleading, and is not consistent with the publication by Saw et al. Histology and MRI was 18M, IKDC was 24 months. IKDC scores were 24 month follow-up. Statistical difference in age between the groups does not warrant additional study. Would recommend deleting this sentence. Page 5/21, line 54, would recommend changing painful to uncomfortable...if properly anesthetized, there is no significant pain with the procedure, as in the liposuction procedure.

	Page 6/21, line 22, The only problem that I see with your hypothesis is that it has been experimentally demonstrated by Kock L, van Donkelaar CC, Ito K. Tissue engineering of functional articular cartilage: The current status. Cell Tissue Res 2012;347:613-627., that AD MSCs lack expression of TGF-beta type 1 receptor, and decreased expression of BMP-2, 4,and 6 receptors thus factors have to be supplemented for chondrogenic differentiation. Concerned that the regeneration may be hampered without the supplementation by growth factors, how will you deal with this? Page 6/21, line 41: Would you consider doing modified subchondral drilling instead of microfracture, because Saw et al. 2011, demonstrated failures with standard microfracture described by Steadman, and changed the technique to drill the holes 1-2 mm apart instead of 3-4 mm apart. In some patients with the holes being far apart, tufts of type 2 collagen occured, but no coalescence was demonstrated. Materials and Methods Page 8/21, line 15. Correct misspelling of enrolment to enrollment. Page 8/21, line 57. Delete period after the) Page 8/21, line 58. Please provide a reference for the Orebro MSK Pain that has been validated for the knee, if you cannot, you should pick one of the many other questionnaires validated for the knee. This instrument has been validated for persistent back pain Page 10/21, line 7. Although the Saw trial did weekly injections after the surgery, a later study that was performed by Saw, et al., Arthroscopy 2015, looking at the osteotomy patients undergoing the procedure found that the regeneration was significantly improved in patients receiving cells the day of the procedure. Personal communication with Stem Cell Scientists from Cambridge and the Cleveland Clinic seem to think the window of greatest opportunity in administration of cells is within the first 3 days. So my recommendation is that one administration of the cells should be done at the same time that the arthroscopy is completed, and possibly one additional post operative day within 3 days of surgery. Page 10/21, line 13. Lignocaine is highly toxic to stem cells, these postoperative injections can be done after ice pack placement, and or cold spray. Would recommend against using the lignocaine. Ref--Dregalla et. al. Stem Cells Trans Med published online January 16, 2014 Page 12/21, line 43. It would greatly improve the study if a quality of life score were to be included like the SF-36, or EQ5D. Discussion Page 15/21, line 19. One limitation that has been identified is the additional growth factors should be added to adipose derived mesenchymal cells to induce chondrogenic differentiation. This is not mentioned in the submission and should be addressed prior to publication. Additionally, classical microfracture may have a negative impact on results as demonstrated by Saw, et. al., Arthroscopy 2011.

REVIEWER	David Frisbie Colorado State University, Orthopaedic Research Center, Fort Collins, CO USA
REVIEW RETURNED	17-Aug-2015

GENERAL COMMENTS	This is an interesting study that the results of will be welcomed. I have two major concerns: 1. The state hypothesis and goal relate to better hyaline cartilage with MSC vs no MSC however the pivotal outcome parameters will not evaluate this outcome. Further the secondary outcomes are indirect at best at reaching this assessment. 2. Enough information exists in pre-clinical work for the authors to attempt to perform a power calculation even if it is qualified as generated from other species.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name William D. Murrell, MD, MS

Abstract

Would recommend changing line 18 to: Technically challenging, expensive and may not have significant improvement over microfracture alone.

Response : I have updated.

Would recommend expanding line 23 to include the source of cells: culture expanded……

Response : unable to change due to word count restrictions

Line 29 would recommend changing statement in parentheses to (microfracture only or microfracture alone)

Response : updated

Line 30 would recommend changing statement in parentheses to (injected adjuvant culture expanded adipose derived stem cells administered post-operatively)

Response : unable to add due to restriction on word count.

Comment-Line 39 - The cartilage regeneration may be underwhelming at 12 months, however from a scientific follow-up perspective this is acceptable. Caution-however, if the regeneration is underwhelming in this time frame, the risk of demonstrating experimentally that the great expense of using adjuvant stem cells to improve regeneration is certainly a risk.

Response : I agree. I have corrected this with :
- “IInitial outcome follow up for publication of results will be at 12months. Further annual follow up to assess long term differences between the two group will occur.”

Background

Line 28-recommend including additional reference with reference 5: Failures, re-operations, and complications after autologous chondrocyte implantation--a systematic review.

Harris JD, Siston RA, Brophy RH, Lattermann C, Carey JL, Flanigan DC.

Osteoarthritis Cartilage. 2011 Jul;19(7):779-91. doi: 10.1016/j.joca.2011.02.010. Epub 2011 Feb 17.

Review.

PMID: 21333744 [PubMed - indexed for MEDLINE]

Response : added. Thankyou.

Page 5/21, line 22 recommend adding additional paragraph: Proof of concept as far as cartilage repair or regeneration requires 3 main requisites. This requires demonstration of adequate production of proteoglycan, predominance of type II collagen, and cartilage structural reconstitution.

Response :
- I have added this later in the text. – See Outcome Measures

Page 5/21, Line 29 delete: (containing bone marrow derived MSCs) as the marrow aspirates were not characterized in this study, and MSCs may or may not have been present.

Response : amended

Page 5/21, line 30 insert: Enhanced tissue repair (with increased proteoglycan production histologically, following….)

Response : amended

Page 5/21, line 38 delete microfracture—in the report it is clearly modified subchondral drilling, and Pridie method is referenced in the original article.

Response : amended

Page 5/21, line 39 change 12 to 18 months. Only postoperative outcome that was recorded at 12 months was IKDC score. Biopsies and MRI were done at 18 months.

Response : amended

Page 5/21, line 41, this sentence needs to be modified. Better to say that additional work is needed because of multiple treatments are confounding variables found in this study, i.e., HA and PBPCs. Would not because of the age difference between the groups. This statement is misleading, and is not consistent with the publication by Saw et al. Histology and MRI was 18M, IKDC was 24 months. IKDC scores were 24 month follow-up. Statistical difference in age between the groups does not warrant additional study. Would recommend deleting this sentence.

Response : amended

Page 5/21, line 54, would recommend changing painful to uncomfortable...if properly anesthetized, there is no significant pain with the procedure, as in the liposuction procedure.

Response : ammended

Page 6/21, line 22, The only problem that I see with your hypothesis is that it has been experimentally demonstrated by Kock L, van Donkelaar CC, Ito K. Tissue engineering of functional articular cartilage: The current status. Cell Tissue Res 2012;347:613-627., that AD MSCs lack expression of TGF-beta type 1 receptor, and decreased expression of BMP-2, 4,and 6 receptors thus factors have to be supplemented for chondrogenic differentiation. Concerned that the regeneration may be hampered

without the supplementation by growth factors, how will you deal with this?

Response :
- I agree that there is considerable debate as to the chondrogenic potential of MSCs from various tissues. De Ugarte (De Ugarte et al 2003) suggests little difference between BM and Adipose derived MCSs in potential which is in contrast to the paper by Kock.
- We hypothesize that the GFs released through the method of micro# with resultant bleeding will assist in MSC differentiation.
- As most papers indicate that the MSCs and even chondrocytes within ACI techniques do not remain within the joint / defect we also anticipate that it is through a paracrine stimulation rather than direct differentiation that the MSCs will act.
- this has been added to the `Background'.

Page 6/21, line 41: Would you consider doing modified subchondral drilling instead of microfracture, because Saw et al. 2011, demonstrated failures with standard microfracture described by Steadman, and changed the technique to drill the holes 1-2 mm apart instead of 3-4 mm apart. In some patients with the holes being far apart, tufts of type 2 collagen occured, but no coalescence was demonstrated.

Response :
- within Australia micro# rather than subchondral drilling is the accepted technique performed by orthopaedic surgeons. To assist in recruitment we chose not to control the micro# technique. Whilst it is a weakness of the study and the surgical technique may effect outcome we hope also to show that this is indeed a strength and that outcome is improved independent of the surgeon and micro# technique used.
- see addition to "Treatment Protocols" –
Treatment Protocols
Arthroscopic Microfracture
All participants will undergo a planned arthroscopic microfracture for an isolated chondral lesion. The technique used for microfracture will be dependent upon the surgeon performing the procedure. Whilst this may potentially affect outcome and is an accepted limitation it has been purposefully chosen to allow recruitment of participants from across the orthopaedic community.

Materials and Methods

Page 8/21, line 15. Correct misspelling of enrolment to enrollment.

Response : corrected

Page 8/21, line 57. Delete period after the)

Response : corrected

Page 8/21, line 58. Please provide a reference for the Orebro MSK Pain that has been validated for the knee, if you cannot, you should pick one of the many other questionnaires validated for the knee. This instrument has been validated for persistent back pain.

Response :

- whilst formerly known as the Acute Lower Back Pain Screening Questionnaire the OREBRO MSK Pain Questionnaire is an accepted and validated questionnaire for other MSk disabling conditions. We

have used it in conjunction with knee specific questionnaires (ie. KOOS) due to its predictive value in regards to `return to work', and potential `recovery'.

Page 10/21, line 7. Although the Saw trial did weekly injections after the surgery, a later study that was performed by Saw, et al., Arthroscopy 2015, looking at the osteotomy patients undergoing the procedure found that the regeneration was significantly improved in patients receiving cells the day of the procedure. Personal communication with Stem Cell Scientists from Cambridge and the Cleveland Clinic seem to think the window of greatest opportunity in administration of cells is within the first 3 days. So my recommendation is that one administration of the cells should be done at the same time that the arthroscopy is completed, and possibly one additional post operative day within 3 days of surgery.

Response :
- I have had similar correspondence with Kay Yong Saw as he has found reduced benefit with initial patients who underwent the SC injections at 4weeks post arthroscope. As the micro# is external to the study it may be performed by any orthopaedic surgeon at any number of hospitals. This creates issue with both SC transport but also requirement to have individual ethics committee approval at these numerous hospitals for SC treatment. As such we chose to do the initial SC injection at 1 week post arthroscope which was in accordance with previous advice from KY Saw.

Page 10/21, line 13. Lignocaine is highly toxic to stem cells, these postoperative injections can be done after ice pack placement, and or cold spray. Would recommend against using the lignocaine. Ref--Dregalla et. al. Stem Cells Trans Med published online January 16, 2014.

Response :
- I agree regarding the toxicity of LA. The LA however is only infiltrated to the subcutaneous layer (under ultrasound guidance) and is not injected intra-articularly.

Page 12/21, line 43. It would greatly improve the study if a quality of life score were to be included like the SF-36, or EQ5D.

Response :
- this is a very good suggestion. Unfortunately as recruitment and treatment has commenced this cannot be added. We will add this score however to current online questionnaires and will collect it prospectively from now. Thankyou for this suggestion.

Discussion

Page 15/21, line 19. One limitation that has been identified is the additional growth factors should be added to adipose derived mesenchymal cells to induce chondrogenic differentiation. This is not mentioned in the submission and should be addressed prior to publication. Additionally, classical microfracture may have a negative impact on results as demonstrated by Saw, et. al., Arthroscopy 2011.

Response :
- these limitations have now been addressed within both the `Background' and "Methods/Design".

Reviewer: 2
Reviewer Name David Frisbie
Institution and Country Colorado State University, Orthopaedic Research Center, Fort Collins, CO USA

Please state any competing interests or state ‘None declared’: Interest Owner in a Veterinary Stem Cell Business

Please leave your comments for the authors below
This is an interesting study that the results of will be welcomed. I have two major concerns:
1. The state hypothesis and goal relate to better hyaline cartilage with MSC vs no MSC however the pivotal outcome parameters will not evaluate this outcome. Further the secondary outcomes are indirect at best at reaching this assessment.

Response :
- I agree with this comment and that histological analysis would be the most reasonable and accepted way to assess hyaline like cartilage repair. Repeat, non therapetuic arthroscopy however was felt to be an unacceptable risk and a barrier to ethics approval.
- MRI T2 cartilage mapping has evidence of ability to indicate cartilage quality though as it is still accepted as a `novel’ technique it was felt that it was not appropriate to be a primary outcome measure.
- the limitation of non-histoligical analysis has been added to the paper : see “Outcome Measures’ –
- “Whilst it is accepted that histological analysis would provide best evidence of quality of tissue regeneration, repeat non-therapeutic arthroscopy for the purpose of performing a biopsy was felt to be both an obstacle to ethics approval and participant recruitment and hence not included in the study design. “

2. Enough information exists in pre-clinical work for the authors to attempt to perform a power calculation even if it is qualified as generated from other species.

Response :
- Our study aims to enrol 40 participants (20 in each group). As a pilot study, it is not intended to be fully powered to detect all important effects. For example, to provide 80% power to detect a "moderate" sized effect (standardised mean difference = 0.5), we would require approximately 141 participants allowing for a 10% loss to follow-up (Machin et al 2009). Given the limited data currently available relating to the effects of autologous mesenchymal stromal cells on knee cartilage defects, our pilot study will provide more specific data on our target population allowing for more precise sample calculations in the future.
- We have done a power calculation on the KOOS outcome score (which has a minimal clinically important change documented as 10points) the required total sample size (allowing for 10% loss to followup) would be 80 (40 per group).
o as KOOS is not our only outcome parameter it is expected that the sample size would be different for the other outcome scores.
o unfortunately funding does not allow for a larger sample size and therefore this study is restricted to being a pilot study.