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Dear Reviewers,

We are most appreciative of your email response regarding precise adjustments to our recent submitted paper: "Orthobiologics in the Treatment of Hip Disorders". We are deeply thankful and proceeded the language polishing, as demanded. Hereafter we kindly share further corrections as suggested:

Reviewer: "The theoretical advantages of orthobiologics are minimal invasiveness, greater healing etc": What do the authors mean with "minimal invasiveness"?. The collection of adipose tissue or of bone marrow tissue can be considered a "minimal invasive" procedure? I think that this concept applies to some but not all orthobiologics.

Answer, in text: The theoretical advantages of orthobiologics are minimal invasiveness (compared to more austere open or endoscopic forms of traditional orthopedic surgery), greater healing potential (than mere exercise or physical therapy, for instance), faster recovery and reduced cost opposed to surgery, making it a viable alternative.

HA:

"It may be produced from animal sources (avian) or …..": are the authors sure that HA only animal source is avian?

Answer: current options attain basically from avian origin (either rooster crest or poultry claw) or bacterial fermentation (*Streptococcus zooepidemicus*; there is current research on *Lactobacillus sp* also). Umbilical cord products have been used, but are not a commercially available option for HA extraction nowadays.

"As an example, there is the combination of calcitonin, dextrose, platelet-rich plasma …..": The sentence should be rewritten. Here the concept should be" a combination with calcitonin etc has been proposed". Furthermore, references should be added.

Answer, in text: For example, the combination of calcitonin [23], sorbitol [24], platelet-rich plasma (PRP) [25,17] and/or BMAC (Bone Marrow Aspirate Concentrate) [26,27] have been postulated.

PRP:

“PRP was used alongside hip arthroscopy surgery for a variety of pathologies….”: “was used” or instead is used?

“The technique consists in the administration of 4.5 mL of PRP into the repaired hip….” - here is not clear if the volumes proposed for the therapy are a standard, are instead derived from a paper, or are the results of the personal experience of the authors. Could you please explain and introduce some reference if necessary?

Answer: the reference was already there at 33, but we have highlighted yet again in the beginning an in the text.

PRP was used alongside hip arthroscopy surgery for a variety of pathologies **[33]**.

The technique consists in the administration of 4.5 mL of PRP into the repaired hip joint capsule in the peripheral compartment through the arthroscopic cannula, as well as 10 mL of PRP in the surrounding soft tissues **as suggested by Marc Philippon and Robert Laprade’s group.** Growth factors present in PRP in addition to all the aforementioned healing potential also aid in postoperative hemostasis **[33]**.

“PRP can also be used in combination with different orthobiologic products, such as HA and bone marrow” - Should “BMAC” be used here instead of "bone marrow”?

Ajusted answer in text: PRP can also be used in combination with different orthobiologic products, such as HA and bone marrow preparations [23-27].

“With this technique, we obtained 3-5 fold platelet concentration increase and 2-4 fold white blood cell from baseline.” - Do the authors mean 2-4 fold increase regarding to white cells? i.e are they using L rich-PRP? This should be clear to the reader.

Answer: regarding the Buffy-coat content explanation might be elusive, we have added two additional clarifications in text: "In our clinical practice, after a standardization protocol for PRP therapy, we opted for handmade PRP preparation, with double centrifugation, enriched with buffy-coat (i.e., Leukocyte-Rich PRP) using the modified protocol of Amable et al. [37, 38]. With this technique, we obtained 3-5 fold platelet concentration increase and 2-4 fold white blood cell from baseline (therefore Leukocyte Rich).

“PRP can usually be combined with HA, or even bone marrow aspirate concentrate (BMAC), which can improve pain and function of the injured hip.” - This concept has

already been introduced before.

Answer: we have humbly extrated it from the final draft

BONE MARROW:

In my opinion, the section entitled Bone marrow is over-simplifying the field.

In particular, statements as "HSCs are thought to be the true drivers for enhancing cartilage and bone regeneration" should be discussed more clearly and extensively.

Answer: We are in agreement with your suggestion. The paragraph was changed for: " The HSCs are thought to be the true drivers for enhancing cartilage and bone regeneration, with an important role in the direct conversion to stromal MSCs and orchestration of the bone formation [40] as described by Omsted-Davis in 2003, who pointed out that a Side Population of marrow stem cells could regenerate the hematopoietic compartment of lethaly irradiated mice when transplanted and could also differentiate to osteoblasts through a mesenchymal intermediate [41]. This effect has also been demonstrated clinically in the study of Marx et al in 2014 where he devised a study with 40 adults in Craniomandibular reconstruction. Those patients attaining to the group with higher CD 34+ counts registered a 100% regeneration of implantable bone, as opposed to 40% of such an effect, when CD34+ were much lower. [42]. Pettine also demonstrated improved results when published a paper on intradiscal injection of Bone Marrow Concentrate with three-year follow-up, recognizing that HSC (CD34+) also played an immunomodulatory role similar do MSCs and reported than patients that received greater concentrations of progenitor cells (Colony Forming Unit - Fibroblast, CFU-F, and CD34+ lineage) experienced faster and greater pain reduction. The authors also claim:"This is the first study to link a clinical improvement to CFU-F and CD34+ cell concentrations in BMC (Bone Marrow Concentrate)"[43].

References:

The same applies to the statement that "non-cultured cells presents some advantages…..". This is a matter of active discussions and many studies have tried to address the issue. The cited reference is not particularly recent. New references should be added to support this observation.

Answer: We are in agreement with your suggestion. The paragraph was changed and a more recent literaturewas included, the final paragraph is: "Expansion techniques risk cell rendering with variable differentiation capacity, increased senescence markers

and tumor degeneration liability. Also, these cells must be cultured for two or three weeks – a slow and expensive process that in the clinical practice demands a two-staged tissue implantation surgery. One important issue is that the use of pure, cultured MSC does not contain haematological stem cells, cytokines and growth factors as concentrated bone marrow does [46]. The outcomes of concentrated versus cultured cells should be assessed more carefully, taking in to account the costs, time expenditure and clinical practice suitability [46]".

The iliac crest is a well-known, common site for bone marrow collection, so the sentence "It has already been shown that a pool of MSCs could be obtained from the iliac crest" should be rewritten.

Answer: Previous studies showed some variables that could influence the number of MSCs, such as anatomic harvest site, aspirated volume, age and sex of the patient. Traditionally, MSCs could be predictably obtained from the iliac crest [47].

BMAC:

"In comparison to PRP, there is a significant variation in the final products achieved. Fortier et al. evaluated the constituents of PRP and BMAC, showing a reduction in platelet content and an increase in white blood cell content in BMAC [47]. The differences between these products could represent a different mechanism of action [47]." That PRP and BMAC have different cellular composition is expected. The cited work was mainly aimed to assess repair ability of BMAC in an experimental model. To my knowledge, the work does not report a direct comparison between PRP and BMAC role.

Answer: Ziegler also portrayed a comparison between these products and concluded that BMAC had a significantly higher interleukin 1 receptor antagonist (IL-1Ra) and possibly a more relevant source of anti-inflammatory therapy for osteoarthritis [57].

ADIPOSE-DERIVED TREATMENTS:

What does "the interference of collagenase" mean? "

Answer: It means that the author used collagenase for SVF obtation. This word was

changed in the manucript – In instead of "In 2001, Zuk identified a stem cell population within human lipoaspirates with the interference of collagenase,… " it was changed for: "In 2001, Zuk identified a stem cell population within human lipoaspirates with the use of collagenase,..."

The authors should check reference 63. They explain results from Cuervo et al but the first author is not mentioned. An accurate check of the bibliography is suggested.
Answer: We apologize for our mistake – the reference 63- now 73, was added and all the reference list were checked.

The work by Nava et al, is not described clearly. Reading the sentence, it seems that it is the first case of viable AD-MSC preparation, that of course is not true. It should be clear that the work indeed describes how viable MSCs exhibiting anti-inflammatory properties can be obtained from microfragmented adipose tissue. Furthermore, the characterization and the clinical use of microfragmented adipose tissue has been reported by a number of papers, not cited here.

Answer: We are in agreement with your suggestion. The paragraph was changed adding: "The microfragmented adipose-derived fraction (MFAT) is a mechanical technique that reduces the size of the adipose tissue clusters to eliminate oil and blood residue. The presence of MSCs in significant numbers was demonstrated (77). Nava et al., evaluated the in vitro survival and content of MSCs and anti-inflammatory activity of lipoaspirate and MFAT. It was noted that MFAT exhibited higher amounts of CD31 positive cells – an endothelial marker, and higher numbers of MSC in comparison to lipoaspirate. The release of cytokines was similar in the first week of culture. However, the total amount secreted by lipoaspirate decreased much more rapidly than those produced by MFAT until 28 days of culture. When the MFAT culture medium of early (3-7 days) or late culture (28 days) was added in a monocyte culture it strongly inhibited the inflammatory pattern. The authors concluded that MFAT presents a long-lasting effect due to the anti-inflammatory activity attributed to their MSC content. These cells release cytokines that modulates the inflammatory cascade by a variety of mechanisms [77]".

EXPANDED MESENCHYMAL STEM CELLS

Again: in my opinion, this chapter does not adequately describe the complexity of the subject. It should be improved.

Answer: We are in agreement with your suggestion- the chapter was changed, adding an introduction for mesenchymal stem cells and some articles related to the treatment of hip disorders. The changes were: "Mesenchymal Stem Cells were named almost 30 years ago as a class of cells that could be isolated from a variety of tissues, including bone marrow, adipose tissue, dental pulp and umbilical cord. These cells can be expanded in culture maintaining their in vitro ability to induce a variety of mesodermal phenotypes and tissues, including differentiation into bone, cartilage and fat – showing its multi-potent potential [78] . Dominici et al., 2006 published an article showing that the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a minimal criteria to define human MSC. The criteria includes: Plastic adherence of MSC when maintained in culture conditions; positivity for markers CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79, CD19 and HLA-DR; Differentiation in osteoblasts, adipocytes and chondroblasts in vitro [79]. In 2011, Caplan proposed a change in the name of these cells – Medicinal Signaling Cells (MSC), due to the fact that this is the main role of these cells – the ability to secrete bioactive factors, acting immunomodulatory and trophic and showing the great importance of their paracrine effect. Thus tissue-specific resident stem cells are the responsibles for the construction of new tissue, stimulated by bioactive factors released by exogenously MSC [80, 78].

In 2012, Zhao et al., published a paper with 100 patients treated with core decompression in comparison with patients treated with autologous implantation of culture BMMSC. The follow-up was held until 60 months after procedure. As a result, the authors showed a protective effect in the BMMSC group regarding the progression of the osteonecrosis, since the cell group presented significantly less patients with osteonecrotic stage progression in comparison to CD. It was also verified a decreased quantitative volume in

those patients treated with cells in comparison to CD. The conclusion of the authors is that BMMSC is a safe, reliable and highly effective procedure for the treatment of early-stage ONFH [81]. In 2017, Mardones et al., published a study evaluating safety and efficacy of intra-articular infusion of ex vivo expanded autologous bone marrow derived MSC in patients with OA of hip. Ten patients were injected, each one with a dose of 60 x 106 cells in three consecutive weekly doses. The follow-up period was 16-40 months. Patients showed a significant improvement in the score evaluation in comparison with pre and post-infusion. The radiographic score in general did not change with exception of one patient who had some improvement. The authors concluded that three consecutive injections of expanded BM-MSC proved to be safe and clinically effective treatment in restoration of range of motion and function of the hip [82]".

I think that the paper could be interesting, but a revision of different chapters is needed.