UNIVERSIDAD AUSTRAL

Buenos Aires, May 7th, 2018

Editor in Chief *World Journal of Gastroenterology*

Dear Editor,

It is a pleasure to submit the revised version our work entitled *"Taking advantage of the potential of mesenchymal stromal cells in liver regeneration: cells and extracellular vesicles as therapeutic strategies"* that we submit to *World Journal of Gastroenterology*.

We appreciate very much the opportunity to improve our work with the comments and suggestions raised by the Editor and the reviewers. We have performed a detailed work and responded to all comments.

We now think that our manuscript is suitable for publication in WJG.

Please, do not hesitate to contact me if you need further information regarding this manuscript.

Looking forward to receiving from you.

Sincerely yours,

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Reviewer #1:
The manuscript from Fiore et al. is an interesting review describing the current status of the preclinical and clinical protocols investigating the use of MSCs in liver diseases. Furthermore it focuses on MSC-derived secretome and extracellular vesicles therapeutic potentials. The review is very interesting and updated in its collection and description of all the clinical protocols for liver diseases envisioning the use of either autologous or allogeneic MSCs. I find it worthy of publication quite in its actual form, however I find very difficult to read Figure 2 due to the reduced size of the characters. The authors should improve the output of the Figure for a better understanding.
The quality of figure #2 was improved as requested.

Reviewer #2:
Manuscript titled "Taking advantage of the potential of mesenchymal stromal cells in liver regeneration: cells and extracellular vesicles as therapeutic strategies." deals an important issue of Cell-based therapies for acute and chronic liver diseases. This review summarizes the latest results achieved in clinical trials using MSCs as cell therapy for liver regeneration, the role of EVs in liver physiopathology and the potential of MSC-derived EVs as intercellular mediators and therapeutic tools in liver diseases. The work is good, updated, interesting and fluent. Moreover, there are some minor revisions to be addressed before to accept it for publications.

Please strengthen, improve and update better the introduction section adding more details and interesting information on:
• (first paragraph) non-alcoholic fatty liver disease: 4Ps medicine of the fatty liver: the research model of predictive, preventive, personalized and participatory medicine-recommendations for facing obesity, fatty liver and fibrosis epidemics. EPMA J. 2014 Dec 7;5(1):21. Echocardiography and NAFLD (non-alcoholic fatty liver disease). Int J Cardiol. 2016 Oct 15;221:275-9. Fatty liver disease and lifestyle in youngsters: diet, food intake frequency, exercise, sleep shortage and fashion. Liver Int. 2016 Mar;36(3):427-33.

This is an important comment. We have included a comment in the *Introduction* to address this point (Page #6) and cited the suggested bibliography:

"Different toxic, metabolic, and inflammatory insults result in liver diseases and generate different degrees of inflammation, apoptosis, and necrosis of parenchymal cells [1-4]. For example, acute liver failure (ALF) is characterized by a sudden and massive death of hepatocytes that lead to abrupt hepatocellular and systemic dysfunction [3]. Similarly, in patients with chronic liver disease an important loss of viable parenchymal cells is also observed [1, 2, 4]. Cirrhosis is caused by diverse chronic liver diseases, such as viral hepatitis and chronic alcoholism[1, 2]. Lately, increases in the prevalence of hypertriglyceridemia, obesity and diabetes in developed countries have resulted in an increase in the incidence of non-alcoholic fatty liver disease (NAFLD)[4, 5]. This condition is characterized by a lipid accumulation in the liver that could lead to hepatocytes apoptosis and inflammation, and different degrees of fibrosis (non-alcoholic steato-hepatritis or NASH). Regardless the origin of chronic liver disease, hepatocytes apoptosis results and extracellular matrix accumulation produced by activated hepatic stellate cells will affect liver histoarchitecture and ultimately its function [4]".

• *(second paragraph) different source of MSC such as adipose tissue and other possible tissue engineering applications: Asymmetrical seeding of MSCs into fibrin-poly(ester-urethane) scaffolds and its effect on mechanically induced chondrogenesis. J Tissue Eng Regen Med. 2017 Oct;11(10):2912-2921. Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions. Maturitas. 2014 Jul;78(3):188-98. Mesenchymal stem cells from adipose tissue which have been differentiated into chondrocytes in three-dimensional culture express lubricin. Exp Biol Med (Maywood). 2011 Nov;236(11):1333-41.*

This is other interesting comment which support the broad advantages of MSCs for therapeutic purposes. IN the new versión of the manuscript we included the suggested papers in order to address this point (Page #5 and #9).

Please improve the conclusion section: please specify the clinical relevance of your work, the rational of this work, innovation, limitations, your critical view and some important suggestions for the scientific community.

This is a very important suggestion that will increase the strength of our work. We included a comment in the *Conclusion section* to address this point (Page #20):

"MSCs-based therapy has emerged as a potent and innovative treatment for acute and chronic liver diseases. The safety and feasibility observed in the early clinical trials using MSCs have increased the interest to translate the use of these cells to the clinic. Moreover, pro-regenerative results and an improvement in the life quality of patients were observed. In ALF, MSCs could have a role decreasing liver damage progression due their immunomodulatory properties. In chronic liver diseases, MSCs could contribute to decrease liver damage and to ameliorate the degree of fibrosis. Even more, in both case MSC treatment could not only delay the transplant but also to avoid it in some particular cases. In addition, in the post-transplant setting, MSC therapy could extend the graft survival and/or decrease the amount of immunosuppression required. Although the main mechanism by which MSCs support the repair and regeneration of injured livers is by releasing paracrine factors, strong evidences demonstrated that this paracrine mechanism is mediated by EVs released by MSCs. Therefore, due to EVs' stability for long periods of time and easy isolation methods they have become a therapeutic option to MSCs treatments in liver diseases. At present, EVs are strongly explored for therapeutic or diagnostic application, and more information is needed to develop more efficient tools for liver diseases based on MSC-EVs. However, it is important to understand that therapeutic potential of MSCs or its EVs is still a matter of debate. In addition, standardization of source of MSCs, culture conditions, pre-condition protocols for cell transplantation, administration route, doses and time of treatment is required. Nevertheless, considering that development of new therapeutic approaches for liver diseases is urgent, MSCs emerge as potent innovation. Thus, take advantage of the therapeutic potential of MSCs as promising tool for liver regeneration could attend to an important worldwide human health problem".

Reviewer #3:
In the manuscript "Taking advantage of the potential of mesenchymal stromal cells in liver regeneration: cells and extracellular vesicles as therapeutic strategies" the authors reviewed the literature about the use of mesenchymal stem cells (MSCs) and

extracellular vesicles (EVs) released by MSCs in liver diseases and regeneration. The topic is very interesting but recently (2017-2018) many reviews have been published concerning this subject, some of which are not reported in the references. In my opinion the proposed manuscript does not add anything new to what is in the literature.

We appreciate this comment. However, we believe that this review will contribute to the scientific community in general, and in particular to those who are working on liver regeneration and MSCs. Although other reviews were published none of them cover the clinical use of MSCs in liver diseases, the latest clinical trials, mechanisms by which MSCs exert their therapeutic effect, and the role of EVs as mediators of this effect. In our review a number of works published during the last two years have been included.