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LETTER TO THE EDITOR

Reply: Standardized Procedure for Bone Marrow MSCs Preparation for Clinical Use

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First of all we would like to thank Huang and colleagues for their interest in our work and their valuable comments [1]. Human mesenchymal stem cells (MSCs) represent a great potential for medicine [2]. In our recently published study, we compared the effect of long-term cultures on genome stability of MSCs from adipose tissue (ADSC, Adipose stem cells) and bone marrow (BM-MSC) produced for therapeutic purposes [3]. Consequently, MSCs were cultured according to previously developed Good Manufacturing Practices (GMPs) processes that have been fully validated through two European Consortium, that is, CASCADE (FP7-HEALTH-233236) and REBORNE (FP7-HEALTH-241879), and are currently used in clinical trials. We focused on the use of platelet lysate (PL) containing media in comparison with standard culture conditions employing the commonly used fetal calf serum (FCS) containing media and evaluated the impact of two oxygen tensions, that is, normoxia (21% O_2) and hypoxia (1% O_2).

For the PL-based culture conditions, ADSCs were maintained in 2% PL and BM-MSCs in 4% [4–6]. By comparison to a more classical FCS containing media, PL-containing media improved MSC growth and it has obvious benefits regarding safety [7]. It must be stressed that under the PL concentrations used in our study, that is, ADSCs (2%) and BM-MSCs (4%), ADSCs and BM-MSCs reached the same cumulative number of population doublings at the end of passages 1 (≈8–10 CPDs, Cumulative Population Doubling) and 4 (≈17–20 CPDs). Therefore, we were able to evaluate the DNA damage sensing and repair, according to the oxygen tension, in MSCs with comparable CPDs and passages.

For the oxygen tension, we used 21% and 1% O_2, which are markedly different. The oxygen tension in the bone marrow is below 2% outside the vessels indicating that 1% oxygen should be considered as a relevant hypoxic setting. As suggested by Huang and colleagues, higher oxygen tensions (5-6%) could be more beneficial for ADSCs as it would be closer to the oxygen tension of their niche; however, no *consensus* has been found to reproduce accurately the in vivo O_2 concentrations in culture, since even short or longer culture periods for a same oxygen tension are supposed to induce different cell responses from stress to cell death [8]. New studies need to be performed to verify this hypothesis. Importantly, besides their original niches, MSCs implanted in the core of tissue-engineered constructs for regenerative medicine will encounter very low O_2 pressures until these implants become fully vascularized leading to recent studies assessing the impact of near anoxia conditions (0.1% O_2) on MSC paracrine functions [9]. The key role of their immunosuppressive activity on the clinical efficacy of MSC paves the way for the study of hypoxia effects on the capacity of MSCs to inhibit T, B, and NK cell proliferation [10]. As mentioned by Huang and colleagues, we agree that some standardization for the culture of MSCs will be necessary [11]. Our study is part of a larger effort addressed to better comprehend the effects of long-term culture using GMP procedures. One of our aims is to characterize accurately the various culture conditions delaying or reducing the phenotype loss in MSCs, which was partly verified as observed with the differentiation potential of our studied cells under hypoxic conditions.

Thus, new data will be available soon regarding the immunomodulatory potential, secreted factors as well as other features of genomic stability including the replicative stresses. Because such studies are expansive, require a large set of expertise, some international consortium could be set up to help design and perform the most pertinent and comprehensive experiments to quickly propose standardized long-term culture conditions.

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Author Contributions

N.B., K.T.: manuscript writing and final approval; L.S.: manuscript writing; R.P.: manuscript writing, editing and final approval.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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