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A relativity concept in mesenchymal stromal cell manufacturing

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Abstract

Mesenchymal stromal cells (MSCs) are being experimentally tested in several biological systems and clinical settings with the aim of verifying possible therapeutic effects for a variety of indications. MSCs are also known to be heterogeneous populations, with phenotypic and functional features that depend heavily on the individual donor, the harvest site, and the culture conditions. In the context of this multidimensional complexity, a recurrent question is whether it is feasible to produce MSC batches as "standard" therapeutics, possibly within scalable manufacturing systems. Here, we provide a short overview of the literature on different culture methods for MSCs, including those employing innovative technologies, and of some typically assessed functional features (e.g., growth, senescence, genomic stability, clonogenicity, etc.). We then offer our perspective of a roadmap on how to identify and refine manufacturing systems for MSCs intended for specific clinical indications. We submit that the vision of producing MSCs according to a unique standard, although commercially attractive, cannot yet be scientifically substantiated. Instead, efforts should be concentrated on standardizing methods for characterization of MSCs generated by different groups, possibly covering a vast gamut of functionalities. Such assessments, combined with hypotheses on the therapeutic mode of action and associated clinical data, should ultimately allow definition of in-process controls and measurable release criteria for MSC manufacturing. These will have to be validated as predictive of potency in suitable pre-clinical models and of therapeutic efficacy in patients.

Key Words: *cellular therapy, clinical trial, manufacturing, MSCs*

Introduction

The clinical use of mesenchymal stromal cells (MSCs) for tissue regeneration, immune modulation or graft enhancement has reached dimensions deserving serious consideration and critical discussion by the scientific and clinical communities [1]. Indeed, even high-profile journals have provided the forum for controversial debates, either challenging the soundness of putative therapeutic modes by MSCs or advocating their legitimate clinical experimentation despite the absence of demonstrated biological mechanisms [2]. Beyond the extreme positions taken by opposite fringes, it is becoming apparent that the field requires well-designed randomized, prospective, controlled trials that deliver quantitative outcome measures, on the basis of which it will be possible to verify or reject specific hypotheses.

To enable such trials, manufacture of MSC batches becomes of primary importance. It is necessary at this stage to make a distinction between those trials in which a "small-scale" production of MSCs is sufficient, for example, in an autologous use setting, from those which require the manufacture of 10 000 or more doses, typically for allogeneic transplants, in which large-scale production models are likely key to economic sustainability. Obviously, the latter can only be based on extensive expansion of MSCs, which in turn opens several questions about preservation of function and onset of senescence.

MSC preparations are considered advanced therapy medicinal products by European regulation (European Commission [EC] 1394/2007). In the United States, they are considered a more-than-minimal-manipulated cellular and gene therapy product regulated under section 351 of the Public Health Service Act (42 U.S.C. 262). In Europe, MSCs as advanced therapy medicinal products require an authorization of national regulatory authorities from the countries involved in a clinical trial. In the United States, for conducting clinical trials using MSCs, it is mandatory to have an approved Investigational New Drug Application from the Food & Drug Administration. Although some differences exist between

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Europe and the United States, in both cases, MSCs should be produced according to Good Manufacturing Practice (GMP) rules, associated with the requirement to define suitable release criteria and/or potency assays.

In this context, the purpose of the present article is to offer a concise perspective on the parameters to be considered in the production of large MSC batches and how these can be modulated by specific culture systems and operating conditions. A strategy is then proposed to define manufacturing processes integrating scientific developments and clinical perspectives with prospected industrial exploitation, in compliance with regulatory pathways.

Onset of senescence during MSC expansion

When MSCs are transferred from their native niche to a polystyrene culture dish, they change from small, mostly quiescent cells, to spindle-shaped, actively proliferating cells (Figure 1). At early passages, the cells divide about once a day, which contributes to their popularity for therapeutic purposes. It should be realized, however, that expansion comes at a price. As their time in culture progresses, their proliferation rate declines, and depending on the donor and culture conditions, MSCs enter a state of replicative senescence after 20–30 cell divisions [3–5]. During this process, MSC morphology changes from relatively small spindle-shaped cells to larger and flattened cells, with typically more pronounced actin cytoskeleton fibers. Concomitant with the process of senescence, MSCs tend to progressively lose their multi-potency. Especially well-documented effects are the loss of *in vitro* differentiation into the osteogenic, chondrogenic and adipogenic lineages after culture expansion, which occurs at an earlier population doubling than senescence itself, and does not occur at the same time for all lineages [6,7].

Loss of multi-potency and onset of senescence indicate that MSC properties change during culture expansion. Surprisingly, the conventional panel of cluster of differentiation (CD) markers used to characterize MSCs is only marginally affected. Thus, although the typical CD "signature" can be used to confirm the mesenchymal stromal nature of MSCs (i.e., positivity for CD73, CD90 and CD105) [8], surface markers correlating to MSC function and multipotency are largely lacking [9]. The expression of several factors such as alkaline phosphatase and STRO-1 does decline after culture expansion, but is not reliably predictive of differentiation capacity or other immunomodulatory properties [10].

MSCs are telomerase-negative cells and culture expansion is associated with a decrease in telomere length, which begins at the start of culture [11]. A number of immortalized MSC lines have been reported [12–15], exhibiting maintained multi-potency and thus suggesting a causal relationship between the two. Culture expansion has also been implicated in issues related to genomic stability. MSCs accumulate DNA damage during expansion, and an increase in DNA adducts such as 8-oxo guanine have been reported [6,16]. Moreover, after about 15 population doublings,

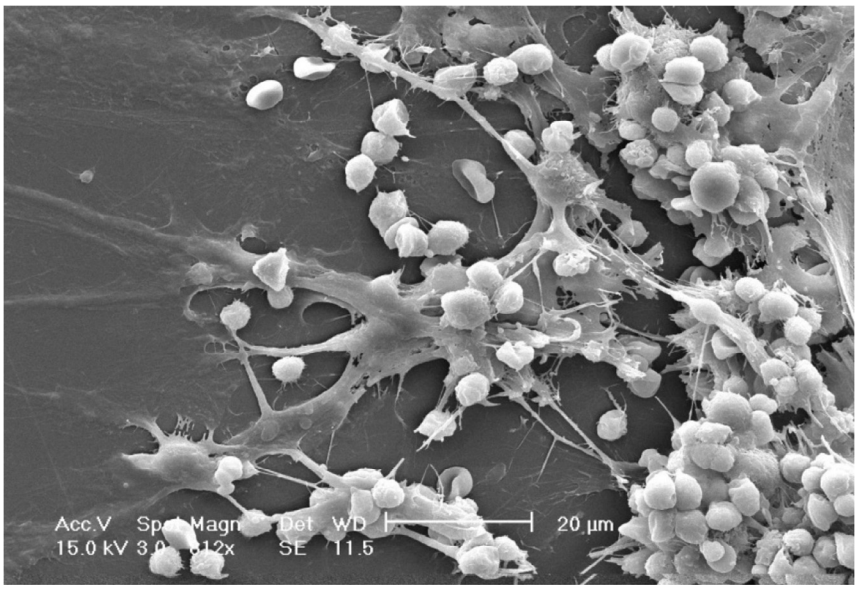

Figure 1. Changes in MSC morphology. Scanning electron microscopic image of a human bone marrow biopsy, 24 h after seeding onto tissue culture polystyrene. The image depicts the different shapes of cells, from small and rounded representing the native shape in the bone marrow, to cells that recently attached to the surface and large flat cells, which have spread and will start proliferating. Some non-adherent, round cells are likely of hematopoietic origin, including red blood cells displaying a concave morphology.