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Pooled human serum: A new culture supplement for bioreactor-based cell therapies. Preliminary results

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Abstract

Background aims. Bone marrow mesenchymal stromal cells (MSCs) are an appealing source for several cell-based therapies. Many bioreactors, such as the Quantum Cell Expansion System, have been developed to generate a large number of MSCs under Good Manufacturing Practice conditions by using human platelet lysate (HPL). Previously, we isolated a novel cell population in human bone marrow, mesodermal progenitor cells (MPCs), which we identified as precursors of MSCs. MPCs may represent an important cell source for regenerative medicine applications. Because HPL gives rise to a homogeneus MSC population, limiting the harvesting of other cell types, we investigated the efficacy of pooled human AB serum (ABS) to provide clinically relevant numbers of both MSCs and MPCs for regenerative medicine applications by using the Quantum System. *Methods.* Bone marrow aspirates were obtained from healthy adults undergoing routine total hip replacement surgery. The aspirates were used to generate primary cultures in the bioreactor. HPL and ABS were tested as supplements to culture medium. Morphological observations, cytofluorimetric analysis and lactate and glucose level assessments were performed. *Results.* ABS gave rise to both heterogeneous MSC and MPC populations. About 95% of cells cultured in HPL showed a fibroblast-like morphology and typical mesenchymal surface markers, but MPCs were scarcely represented. *Discussion.* The use of ABS appeared to sustain large-scale MSC production, as well as promote the recovery of a subset of MPCs, resulting in a suitable alternative to HPL in the cell generation based on the Quantum System.

Key Words: *Bioreactors, bone marrow–derived MSCs, large-scale cell manufacturing, xeno-free supplements*

Introduction

Mesenchymal stromal cells (MSCs) represent a promising tool for cell-based therapies because of their self-renewal, multilineage potential, homing and immunomodulatory properties, in addition to their relative lack of ethical concerns [1–4]. MSCs from bone marrow are being exploited for the regeneration of cartilage, osteochondral tissue and major bone defects, having an active contribution in regenerative medicine [5–7]. Minimal criteria have been defined in the International Society for Cellular Therapy (ISCT) position paper to successfully isolate MSCs: adherence to plastic; specific non-hematopoietic surface antigen expression, including CD105, CD73 and CD90; and *in vitro* differentiation toward osteoblasts, adipocytes and chondrocytes, following standard cell culture conditions [8].

MSCs are usually isolated *in vitro*, taking advantage of their plasticity and ability to adhere to plastic surfaces. However, as reported from Le Blanc *et al.* [3], *in vitro* manipulation can lead to functional and phenotypic changes that make MSCs different from their *in vivo* progeny.

The limited content of MSCs in bone marrow requires an *in vitro* expansion to reach a cell number

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suitable for cell therapy protocols (1–5 million cells/kg of body weight) [9]. Fetal bovine serum (FBS) at 10% is used in many traditional MSC expansion protocols as a supplement to culture medium because its characteristics are routinely pre-screened to guarantee the optimal growth of MSCs and the bio-safety of the cellular product. However, the possibility of immunological reactions in the host due to the xenogeneic proteins has raised some concerns regarding the rejection of the transplanted cells. As result, several countries have legislated restrictions on the clinical grade cellular preparation in which animal-derived reagents are used [10–12].

Cell culture protocols using human blood derivates are being considered at a preclinical level. Autologous serum (AS) has been demonstrated to be equally effective as FBS when added to culture medium for human MSC isolation and expansion [13–15]. MSCs expanded in AS proliferate faster but differentiate more slowly than those expanded in FBS, without losing their characteristic phenotype and differentiation capability [16,17]. These differences are also evident in gene expression. Genes associated with the cell cycle are overexpressed in MSCs cultured in FBS and suggest premature replicative senescence [13,17].

Conversely, opposing data have been reported in literature on the use of AS. Some authors have highlighted that it does not seem to sustain cell proliferation [17,18], whereas other studies have been successful in isolating and expanding MSCs using this supplement [19]. Recently, platelet-derived products are of increased clinical interest thanks to their efficacy in enhancing bone regeneration and soft tissue healing [11]. Human platelet lysate (HPL) was recently demonstrated to be a powerful substitute for FBS in MSC expansion to achieve a high concentration of growth factors [20,21]. It has been demonstrated that 5% HPL is significantly better in terms of clonogenicity and proliferation potential compared with 10% FBS. Moreover, MSCs cultured in HPL show genomic and phenotypic stability and no tumorigenic transformation [11].

In European countries, MSCs are considered advanced therapy medicinal products in compliance with the European Commission regulation 1394/2007 and must be produced under Good Manufacturing Practice (GMP) conditions to guarantee efficacy and safety of the therapeutic product [22,23]. The GMP system ensures that cells are produced under the highest quality controls following standard operating procedures. The transition from the preclinical to clinical phase requires product reproducibility [24]. This objective can be reached by standardizing many parameters, such as medium and culture conditions, which are still laboratory-specific criteria.

To achieve GMP conditions, cells must be cultured in a closed system. New bioreactors have been developed to generate a large number of MSCs under GMP conditions. The Quantum Cell Expansion System is a closed and automated system that releases a human-grade product and generates a final yield suitable for many cell therapy protocols [25]. Bioreactor culture using HPL results in a homogeneous MSC population, limiting the harvesting of other cell types. Interestingly, we previously isolated mesodermal progenitor cells (MPCs) from adult bone marrow and identified them among typical MSCs when cultured in a medium supplemented with either AS and pooled human AB serum (ABS). These cells are quiescent: Ki-67 negative, with long telomeres and express the pluripotency-associated transcription factors Oct-4 and Nanog instead of RUNX2 and Sox9 typical for the MSC phenotype. Phenotypically, they share the expression of CD105 with MSCs but lack expression of CD73 and CD90. This novel population of cells, isolated from BM, may be able to produce fresh MSCs at the site of damage and sustain the generation of endothelial cells in appropriate conditions [26–28].

To obtain a mixed population containing both MSCs and MPCs, AS or ABS may be used instead of HPL. AS appears to be a feasible culture supplement because it allows both recovery of a subset of MPCs and manufacture of a safer product due to its autologous origin [26].

However, AS can be used for only a minority of clinical protocols involving low numbers of MSCs. In these few cases, it is possible to obtain the appropriate volume of peripheral blood. Numerous cell therapies require a large quantity of MSCs, which likewise requires large amounts of culture medium [13]. Moreover, the use of AS is limited by significant serum variability, and it is not be used in the presence of hematological diseases, chemotherapy or infections. For these reasons, we investigated the efficacy of ABS as a potential substitute for HPL and AS for use in large-scale MSC and MPC production using the Quantum System.

Methods

Patient recruitment

Four BM aspirates were obtained from the femoral canal of healthy adult individuals, aged 60–80 years, undergoing routine total hip replacement surgery [29] after written consent, according to the Declaration of Helsinki and the local ethics committee (Comitato Etico Sperimentazione Farmaco CESF, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; file no. 27880/2016). Each BM sample was used to generate MSCs in both bioreactor and tissue culture flasks (see online supplementary Methods).