Cell Culture Technologies

Principles of *in vitro* nutrition for human mesenchymal stromal cells

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Introduction

A timely clinical application of stem cell therapies has been expected for years. Although stem cell research is developing rapidly, stem cell culture is still associated with significant problems regarding phenotypic and functional characterization in complex, chemically undefined culture media. In the cultivation of mesenchymal stromal cells, components of animal origin and/or chemically undefined extracts like human platelet lysate are still used for the formulation of culture media because the quality of the most common basal media is not adequate for cell culture in chemically defined environments. Experimental evidence shows that the better the qualitative and quantitative composition of the basal medium, the fewer additives of animal origin or chemically undefined components are needed to obtain functional human stem cell cultures.

Objectives

This contribution deals with essential nutritional aspects concerning cell culture media for clinical translational research. It describes the development of physico-chemically defined cellular micro-environments that modulate function of the metabolism of human mesenchymal stromal cells from various tissues, e.g. adipose tissue.

Methodology

The development process consists of three parts. The first phase focuses on the development of fully defined cellular microenvironments capable of satisfying the essential physiological needs of the cell. For this purpose, the essential chemical elements summarised in Table 1 must be present in the basal culture medium.

Methodology (continued)

Most abundant in all organisms	Less abundant but found in all organisms	Present in small amounts in all organisms
C, H, N, O	Ca, Cl, Mg, P, K, Na, S	Co, Cu, Fe, Mn, Zn

Table 1. Key elements found in all living organisms (Mathews *et al.*). These elements constitute the essential nutrients that must be present in all basal media for the culture of human cells *in vitro*.

The second phase involves a detailed analysis of the physical (pH, osmolality), chemical (compatibility, solubility and stability) and biochemical requirements (energy sources, amino acids, vitamins, precursors of fatty and nucleic acids, inorganic salts, trace elements and chemical impurities) of the cells. Such analysis allows the elimination of complex, chemically undefined variables like fetal bovine serum or human platelet lysate, which are to be considered as critical components in cell culture media.
Thus, (i) the refinement of suitable basal media containing all essential key elements at physiological concentrations, (ii) basic knowledge about the compatibility, solubility and stability of nutrients and (iii) the manufacturing technique of the nutrient mixtures allow the achievement of the objective of the study (Fig.1, 2).

The third and final phase involves the verification of the obtained results by expansion of cells in various culture vessels, including bioreactors (Fig. 3). The latter allow in vitro cultures at significantly higher cell densities and an extended control of the culture conditions by on-line analysis systems for the most important physical culture parameters (temperature, pH, pO2).

Results

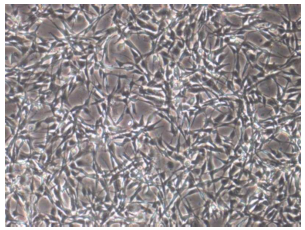

Fig. 1. Human adipose tissue-derived stem cells (hASC) grown for 6 days in xeno- and hPL-free basal medium supplemented with 6 recombinant growth factors. Courtesy of T. Tallone, Cardiocentro Ticino, Switzerland.

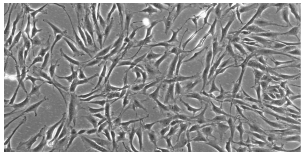

Fig. 2. Immortalized human mesenchymal stem cells (hMSC_TERT) grown for 6 days in a chemically defined protein-free basal medium supplemented with recombinant bFGF as the only growth factor (Salzig *et al.*, 2016).

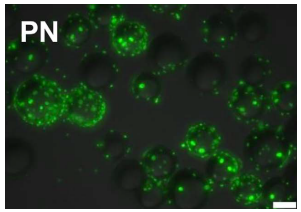

Fig. 3. Immortalized human mesenchymal stem cells (hMSC_TERT) grown for 6 days on microcarriers in a chemically defined basal medium supplemented with recombinant bFGF as the only growth factor. Nuclei are stained with SYBR Green fluorescent dye (Leber *et al.*, 2017).

Conclusion

The results show that knowledge of the principles of *in vitro* nutrition that influence cellular growth processes is essential to develop a chemically defined culture environment. First of all, *all* key elements of living organisms must be present in the nutrient medium. This is trivial in itself, but seems to be underestimated by several scientists who still use inadequate basal culture media such as DMEM or RPMI 1640 to grow human cells. The refinement of the qualitative and quantitative composition of the nutrient mixture represents the next step. At this stage we also have to consider that it is not possible to successfully expand cells by using non-physiological amounts of nutrients. The final step is essential to mimic the natural 3D environment of the cells. On-line control over the most important physical variables contributes to improve the performance of a bioprocess aiming to the generation of cells and sub-cellular particles (exosomes) for regenerative medicine application.
In conclusion, the three-step strategy described here allows the identification of the essential physiological needs of the cells, thus favouring control and modulation of the main metabolic functions. This perspective becomes even more interesting as the omission of chemically undefined and/or expensive basal media supplements in clinical culture processes leads to a reduction of contamination risk and production costs, respectively.

References

- C. Mathews, K. van Holde, K. Ahern, Biochemistry, 3° ed. (2000) ISBN-13: 9780805330663.
- D. Salzig, J. Leber, K. Merkewitz, M.C. Lange, N. Köster, P. Czermak, Attachment, growth, and detachment of human mesenchymal stem cells in a chemically defined medium, Stem Cells Int. 2016 (2016), http://dx.doi.org/10.1155/2016/5246584 Article ID 5246584.
- J. Leber, J. Barekzai, M. Blumenstock, B. Pospisil, D. Salzig, P. Czermak, Microcarrier choice and bead-to-bead transfer for human mesenchymal stem cells in serum-containing and chemically defined media, Process Biochem. 59 (2017), http://dx.doi.org/10.1016/j.procbio.2017.03.017