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Platelet-rich plasma (PRP) as an alternative to fetal bovine serum (FBS) in the culture of mesenchymal stem cells in cell therapy

Abstract

Stem cells (SC) are defined by having proliferation and differentiation properties, with cellular self-renewal capacity, due to these characteristics they are widely studied in the cell therapy field. The Mesenchymal Stem Cells (MSCs) represent the most studied population of SC, due to their capacity to originate cardiomyocytes, skeletal muscle, neural precursors, among other cells. To culture MSCs in the laboratory, culture medium supplemented with fetal bovine serum (FBS) must be used. Despite its extensive use in protocols for cell expansion, FBS presents potential risks that cannot be neglected and are difficult to eliminate from serum. An alternative to the use of SFB is platelet-rich plasma (PRP), which contains high concentration of growth factors (GFs) assisting in cell proliferation *in vitro*. The main objective of the study is to analyze the feasibility of replacing FBS with PRP from umbilical cord blood to supplement the cell culture medium.

Keywords: cell therapy, cell culture, platelets

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Introduction

Stem cells (SCs) are a cell population defined by their proliferation, self-renewal, and differentiation properties. Due to these characteristics, SCs have been extensively studied as a therapeutic alternative in several types of diseases such as heart disease and diabetes. Their features are variable and depend on the degree of differentiation of the population found, they can expand and originate two others identical SCs (symmetric division), originate one undifferentiated cell and another differentiated cell (asymmetric division), or even decrease the population of SCs, generating two differentiated daughter cells (symmetric differentiation division).[1-3]

Cells in the early stages of the embryo that can differentiate into all cells derived from both embryonic leaflets and extra-embryonic membranes are called totipotent.[4] Embryonic SCs (ESCs) derived from the zygote and blastomeres up to the morula stage are classified as totipotent. ESCs are also those derived from the inner cell mass of the blastocyst. However, they have unlimited differentiation capacity and are characterized as pluripotent, that is, with the ability to differentiate into any lineage derived from the three embryonic leaflets, endoderm, mesoderm, and ectoderm, but not from the extra-embryonic membranes. As for the therapeutic use of ESCs, besides ethical issues, there are technical difficulties such as genetic compatibility between donor and recipient and control of tumor formation.

Adult SCs (ASCs) are multipotent, that is, they have a limited capacity for self-renewal and differentiation, giving rise to more specific cell types.[5] ASCs can be isolated from various tissues and are currently the most used in regenerative medicine due to their unique biological properties and do not present ethical impasses related to research like ESCs.[6]

Two main populations stand out among: ASCs hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). MSCs represent the most studied stem cell population and can be isolated from a variety of sources, including, skeletal muscle tissue, adipose tissue, synovial membrane, tooth pulp, cervical tissue, umbilical cord blood, bone marrow, cord lining and perivascular region, amniotic fluid, placenta, lung tissue, liver tissue, and dermal tissue.[6-9] MSCs were first used in experimental cell therapy in 1995 by Lazarus *et al*. Cell therapy with MSCs is an alternative in cases where conventional treatments are ineffective.[10] Currently, the most accepted hypothesis to explain the therapeutic effects of MSCs is an indirect effect through the secretion of cytokines and growth factors, with both paracrine and autocrine effects.[11]

Several studies related to MSC therapy in Phases 1 and 2 are currently in progress. There are also studies in Phase 3 or approved for clinical use. In the culture of MSCs, with most cells maintained *in vitro*, fetal bovine serum (FBS) is routinely used. This is a sticking point that we would like to address. The composition of the FBS can vary subtly with the batch used, however, these variations can change the cell response. This has always been a source of attention in cell culture. In addition, uncharacterized factors can affect the speed and quality of cell growth *in vitro*, and FBS carries a potential risk of contamination. Mainly mycoplasmas, viruses and endotoxins are difficult to be removed from the serum.[12]

Therefore, the search for a procedure that can be an alternative to FBS to ensure safety in cell therapy is something that has been sought for quite some time.[13] The U.S. Food and Drug Administration (FDA) recently reported that over 80% of 66 investigations into new mesenchymal stem cell drugs used FBS in their manufacturing process. This is undoubtedly a source of concern.

An alternative to FBS may have been presented in the early 1980s, through the first studies with platelet lysates *in vitro*. Platelet-rich plasma (PRP) emerged from this pioneering research. PRP was first tested in the culture of fibroblasts, endothelial cells and tumor cell lines and showed promise.[14] PRP contains a variety of plasma proteins and, unlike serum, contains fibrinogen and coagulation factors that can be activated. With this, it is possible to create a temporary three-dimensional fibrin structure that aids in cell adhesion, migration, and proliferation. In addition, PRP is rich in growth factors.[15] In this work, we will explore the hypothesis of using PRP as a substitute for FBS in the culture of MSCs, mainly for cell therapy.

PRP is very versatile and can be obtained from different sources, such as from blood banks or even from the umbilical cord in pregnant

patients. In these cases, to obtain the PRP fraction from the blood, not very complex routines can be established.

PRP growth factors

Platelets when activated, release granules containing growth factors (GFs) including, transforming growth factor beta (TGF-ß), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGFs) and platelet-derived growth factor (PDGF). Such factors are responsible for maintaining the differentiation potentiality into osteogenic, chondrogenic and adipogenic cells, retention of immunosuppressive activity and the expansive acceleration of cells without loss of multi-potentiality.[13,16] PRP has also been shown to induce proliferation and differentiation of MSCs into osteoblasts, stimulate de novo collagen synthesis, and inhibit degradation of newly formed collagen.[17,18]

Some GFs from PRP, including bFGF, PDGF and TGF-ß stimulate cellular function to wound healing, migration, and proliferation of fibroblasts.[18] GFs released by PRP, can be detected during the early stage of healing.[19] The TGF-β superfamily is one of the most abundant GFs released by PRP and constitutes mediators for differentiation and proliferation of MSCs, in addition to its bone formation capacity, it is an essential growth factor that promotes chondrogenesis, both in vivo and *in vitro*. The main targets of TGF-β are fibroblasts, bone marrow SCs, and pre-osteoblasts.[19,20] As well as stimulating the growth of MSCs, bFGF is directly related to osteogenesis and angiogenesis. VEGF also stimulates angiogenesis. FGF2, a specific factor of the FGF family, is involved in endothelial cell proliferation. PDGF is the main mitogen for MSCs and endothelial cells. IGF is related to osteoblast stimulation, cell proliferation and differentiation.[20]

PRP may represent an alternative to the use of FBS. It was observed that human fibroblasts had a significant cell proliferation with the use of PRP supplemented to 10%.[21] The number of GFs present in platelets changes from individual to individual and may influence the potential effectiveness of PRP. So, for the use of PRP, the divergence of lots and donors, variations in the content of GFs and the possibility of contamination by leukocytes must be considered.[15]

Use of PRP in MSCs

PRP is well described in the literature, as a supplementation in culture media and tissue repair. The use of PRP, stimulated cell growth with adequate morphology, i.e. fusiform and adherence to plastic.[22] The immunophenotyping characterization of MSCs, supplemented with PRP derived from cord blood, were similar with MSCs supplemented with FBS, greater than or equal to 90% for CD73, CD90 3 CD105 markers and less than 1% for blood lineage specific markers such as CD34 and CD45. The final cell concentration, starting from the same plated number, of the medium containing PRP was like FBS, this is important as the use of FBS has been widely questioned during use for *in vitro*, cell expansion prior to use in cell therapy.[15]

More recently, a study investigated the biological action of platelet-poor plasma (PPP) and PRP on MSCs from bone marrow (BM-MSCs). The adipogenic potential of BM-MSCs revealed no obvious change, but the osteogenic capacity of BM-MSCs was increased after PRP treatment. CCK8 assays and cell colony formation assays showed that PRP promoted cell proliferation, while this effect of PPP was not obvious. PPP also showed no influence on the cell cycle of BM-MSCs. On the other hand, the expression of β-galactosidase, a biological marker of senescence, was decreased after PRP treatment. Importantly, PRP increased the activity of several pluripotency marker genes, including Sox2, Sall4, Oct4 and Nanog. PRP also promoted cell migration of BM-MSCs through stimulation of the PI3K/AKT signaling pathway.[22]

A point that seems important to us is that the data in the literature show that PRP can be used either as a substitute for SBF or for inducing the differentiation of MSCs. There is no comprehensive study on this issue. In our opinion, it is necessary to establish a number of platelets, and consequently, a range of growth factors that can be used as a cell growth stimulating agent and another to stimulate differentiation. So, while this standardization is necessary, it also shows the versatility that PRP can present.

Conclusion

Several clinical studies, sensitized and concerned about the use of fetal bovine serum as supplementation of cell cultures, have defined an attempt to replace FBS by PRP. The data are promising. On the other hand, the literature shows a close relationship of PRP and differentiation of MSCs. Then there is the issue of stem cell expansion versus differentiation. In some moments, the expansion of the culture is important and in others, their differentiation may be the most fundamental point. Thus PRP should be handled with care and its range of use should be well standardized.

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Conflicts of interest

Author declares that there is no conflict of interest.

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