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Review Article

Use of Autologous Platelet-Rich Plasma Versus Fetal Bovine Serum in Mesenchymal Stem Cells Culture Media

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Abstract

With the advances in regenerative medicine, research is focused towards biologically safe alternatives to cure pathologies. For this purpose, stem cells are potential candidates. An essential component of stem cell culture media is Fetal Bovine Serum (FBS) which provides necessary growth factors and hormones. Unfortunately, there is an increasing concern about quality of FBS as there are reports of adulteration and route of FBS origin and transportation is not fully traceable. Alternatively, Platelet-Rich Plasma (PRP), an autologous preparation, is used in stem cell culture as it provides similar growth factors as that of FBS. Though, the results with the use of PRP show varying degree of success, still it is getting popular among the cell culturing community because of ease of access, cheaper cost and promising response. In this review, we have described limitation in use of FBS in stem cell culture, preparation and potential of PRP as well as comparative behaviour of mesenchymal stem cells (MSCs) in terms of their stemness, proliferation, differentiation and immunoregulatory properties in both cell culture media components i.e. FBS and PRP.

Keywords: *Platelet-Rich Plasma; Fetal Bovine Serum; Mesenchymal Stem Cell*

Introduction

With the advances in regenerative medicine, trend is shifted towards the use of biologically safe therapeutic remedies to cure pathological disorders specially since the last decade. In this scenario, there has been a significant rise in use of platelet-rich plasma (PRP). It is found to be effective not only to deal with blood loss during cardiac surgery as was its first documented use [1,2] but also used to treat arthritis [3], tendonitis [4,5], wound dressing management [6], chronic wound healing [6-10], bone formation and maturation [11,12], musculoskeletal disorders [5] and other cellular paths. PRP is a blood preparation of autologous origin and is defined by its erythrocyte count, it should be less than 5% [13]; platelet count, it should be about five times higher than the normal physiological values [14]; or significantly higher concentration of platelets [15]. PRP is considered as a preferred preparation than the conventional treatment protocols like hormones and antibiotics due to its promising results and least or no deleterious effects on health. These properties of PRP is the fallout of its mitogenic potential and growth factors which are as high as 7 to 30 times than the normal base line values [16,17]. These growth factors are involved in the angiogenesis [18], chemotaxis, extracellular matrix protein synthesis and degradation [19], *in vitro* cell culture growth and expansion [20] specially of MSCs [21]. Although there are more than 15 commercially available PRP preparation systems that deliver varying degree of platelet concentrations, leukocyte counts, fibrinogen quantity, type of activator etc. [22]. Commonly, PRP is prepared in two-step centrifugation process; where either both of centrifugation conditions are the same [23] or first is the soft spin, to separate plasma from erythrocytes and second is the hard spin, to separate PRP from platelet-poor plasma (PPP) [24]. Similarly,

two types of PRP can be prepared; leukocyte-poor plasma and leukocyte-rich plasma [25]. The former is desirable where least local tissue reaction is required, however, leukocytes are involved in tissue repair and wound healing. Even though, there is not a single PRP preparation protocol, it is important to understand all of them in order to fully appreciate its versatile application.

As discussed earlier, regenerative medicine is preferred to be used clinically which utilizes human and animal cell culture. These cell cultures require certain cell culture conditions and a medium that should support growth and proliferation of these cells. Traditionally, FBS is the most commonly used medium for *in vitro* cell culture growth as it contains required hormones and growth factors [26]. However, FBS is not just limited to a combination of hormones and growth factors that help proliferate and stimulate cell differentiation, but also provide necessary proteins, lipids, carbohydrates, minerals, trace elements, buffering agents against pH and oxidation, antibodies and many unspecified factors [27]. Studies revealed the presence of 1800 proteins [28,29] and 4000 metabolites [30] in the serum.

Retrieval and limitations of FBS

FBS is collected from foetuses. The pregnant cows presented at slaughterhouse are separated and after slaughtering, the foetuses are retrieved. Blood from the live unanesthetized foetuses is then collected using a needle directly from the beating heart which shows quite a great deal of manipulation of a live animal [31]. This raises a question of animal welfare and ethics in research [32,33]. Moreover, there is an unintended risk of contamination, low availability, laborious harvesting process, high cost, poor regulatory mechanisms both in research and production industry as well as varying composition makes it less likeable to be used in *in vitro* cell culture studies [32-34]. Furthermore, there are deep trepidations regarding the use of serum of animal origin for propagation of human embryonic stem cell (hESC) for cellular therapy as it contains non-human N-glycolylneuraminic acid (Neu5Gc), making FBS unfit for hESC [35,36].

Unfortunately, there is not an established line that can be traced back in true sense to elucidate the origin of FBS as market is slackly structured since very beginning of its commercialization and this is the foremost foundation deceit in its quality. It is reported that a total of 30000 litres of FBS was sold by New Zeeland in the international market while only half of that was collected annually [37]. It opens every possibility of adulteration of FBS with calf serum or adult serum [37]. A report in 2013 published by GE Healthcare (a unit of General Electric Co.) revealed that the FBS batches from 2008 to 2013 were adulterated, i.e. it contained water, adult serum, serum albumin etc. The Food and Drug Administration (FDA) of the USA pointed out that there is nearly 28 thousand litres of FBS in 143 batches which were effected [38]. There is still not any betterment in the situation till 2014 regarding FBS quality [39].

Keeping in view the current scenario, there is a trend and demand of the scientists to replace FBS with a medium which is relatively cost-effective, abundantly available, locally produced, autologous, with broad spectrum of physiological activity and can support *in vitro* proliferation of the stem cells.

Preparation and application of PRP

PRP, which is typically prepared from the freshly collected blood and activated subsequently adopting various methods including freeze-thaw cycling, addition of $CaCl_2$, sonication etc. has shown some good results in cell culture proliferation studies [40-42]. Usually, PRP is produced by individual facilities to cater its own demands, however, some commercial preparations are also available. Researchers are using PRP in various cell based studied including proliferation, differentiation, tissue engineering and matrix synthesis and found satisfactory and, in many cases, better alternative to FBS as platelets contain a number of growth factors as mentioned earlier. PRP is also known for its successful application in soft and hard tissue repair like bone, tendon, muscle, cartilage and skin [43,44]. A number of studies show varying degree of effects of different concentrations of PRP on cell cultures, although 5%, 10%, and 20% concentration of PRP are employed while keeping 10% FBS as control media [45]. Many yard sticks were used like proliferation, quantification of dsDNA, expression of collage type I and II, aggrecan, cell density, culture areas, number and size of colonies, cell adhesion, viability, apoptosis, differentiation (osteo-, adipo- and chondrogenic), maintenance of immunomodulatory properties, extracellular matrix-receptor interaction, protein synthesis, doubling time and growth curve of the cells, alkaline phosphatase activity, chromosomal alteration, and preserving stemness and multipotency of the cells.

Proliferation

MSCs of adipose origin show higher degree of proliferation rate in PRP compared to PPP and FBS at the same concentrations [46]. Similarly, the population doubling time of these cells is significantly higher in PRP, however they show poor differentiation potential in PRP. Moreover, the proliferation of osteocytes, myocytes, and tenocytes increase significantly when cultured in a medium containing PRP with low platelet count, and that of osteoblasts and tenocytes in high platelet and low WBC count, and that of tenocytes in high platelet and high WBC count when compared to either 2% or 10% FBS [43] suggesting that it not only the concentration of the platelets but also that of WBCs account to culture a specific target cells. Similarly, formation of a thicker cartilage with a better equilibrium modulus is observed when chondrocytes are cultured in 20% PRP compared to 20% PPP or 20% FBS [47]. Even the traditional concentration of 10% FBS yields significantly lower cell proliferation compared to 10% PRP [48]. In case of modified adipocytes, 2% PRP concentration was sufficient enough to significantly promote proliferation compared to the same concentrations of PPP and FBS [49]. Still others found contrary results to study the differentiation potential of adipose-derived cells for their capacity of bone formation [50], though there was heterogeneity in their cell samples and PRP.

Differentiation

For differentiation studies, heat inactivated PRP (5% and 10%) was found equally effective for osteogenic and adipogenic differentiation as that of FBS (10%) [51]. Moreover, Minimal Essential Medium (MEM) supplemented with PRP promotes cell differentiation in chondro-, osteo- and adipogenic differentiation and supports culture proliferation [52,53], therefore, a 10% concentration of PRP provides either comparable [54] or better cellular proliferation rates of cell cultures than FBS [55]. Commercially available mesenchymal cells show quite higher expression of osteogenic (RUNX2) as well as chondrogenic markers (Sox-9) when cultured in PRP compared to FBS [48].

Apoptosis

Although there is a little work on comparative study of apoptosis in transplanted tissue, however, accelerated apoptosis is not desirable characteristic. PRP has shown to enhance the anti-apoptotic activities of the modified adipocytes as compared to PPP and FBS because the former significantly reduces TNF-α induced apoptosis [49]. Similarly, mRNA of DAPK1 and Bcl-2-interacting mediator of cell death (BIM) is also significantly reduced.

DNA contents

The antigenic phenotype, including CD73, CD90, CD105, CD14, CD19, CD34, CD45, of cell cultures raised either in PRP and FBS are same [56]. Synthesis of dsDNA quantity is similar in 10% and 20% PRP to 10% FBS [45]. Others, have reported a non-significantly higher DNA contents in 20% PRP compared to 20% PPP or 20% FBS in the chondrocytes [47]. Conversely, others have found a highly significant increase in the DNA contents when the chondrocytes are treated with PRP as compared to PPP or FBS [57].

Metabolites

The amount of collagenase-I as well as aggrecan expression was similar in 10% and 20% PRP to 10% FBS [45]. Even expression of aggrecan is very high when calls are cultured in 10% PRP compared to 10% FBS [48]. Although, some studies reported decreased expression of aggrecan, but both of these vary in terms of using the source of stem cells [58] and the half-life of this proteoglycan [59]. PRP rich medium supports formation of collagen-I and higher expression of aggrecan compared to a medium that lacks PRP [60]. In some studies, collagen-II formation is seen higher in tissues in 20% PRP compared to 20% PPP or 20% FBS [47]. Some researchers are of strong advocate of cell culture stimulatory properties of PRP as they found highly significant rise in the formation of collagens and proteoglycans in PRP compared to their contemporary preparations of PPP and FBS, however, the products are structurally similar of all the treatment groups [57] which indicates that the cells are stable in every case. The glycosaminoglycan (GAG) content is also higher in the chondrocytes formed in 20% PRP compared to 20% PPP of 20% FBS, however, hydroxyproline contents are similar in all media [47]. Expression of some other metabolites like matrix metalloproteinases (MMP) catabolic molecules including MMP-1 and MMP-3 is also enhanced in the presence of PRP [60].

Immunomodulatory properties

The first and foremost advantage of using PRP is that it is not required to screen it in routine to check optimal stem cell growth and safety regarding the host-immune reaction/ rejection which is necessary for FBS. Although, there are not many studies to compared the immunomodulatory properties of stem cell in PRP and FBS, however, some reports indicate enhanced immunomodulation in PRP [46] or no effect at all [61,62].

Preserving stemness and multipotency

The most commonly used criteria to evaluate stemness is to measure matrix metalloproteinase-2 (MMP2) gene. The studies show that, though, the cells grown in PRP and PPP produce multi-fold increase in its expression, however, the higher expression is recorded in FBS [46]. There are a number of studies on the use of PRP suggesting that its influences cell culture in a dose-dependent manner [49], however, the theory of "the more, the better" does not fits here.

Conclusion

PRP has, so far, proved to be a suitable alternative to FBS in many studies. A lot of data is available on preparation and characterization of PRP. There is a need to establish a standardized procedure to prepare and activate it. As plasma contains immunoglobulins that may pose a risk to cells, therefore, concentration of immunoglobulins should be decreased and defined in PRP. With a possible increase in demand of PRP in MSC culture, it is necessary to produce it under Good Manufacturing Practices (GMP). In a nut shell, collaborative efforts by academia and industry are required to understand and utilized full potential of PRP.

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