DOI: 10.22059/ijvm.2018.250527.1004872 *Iranian Journal of Veterinary Medicine*

Comparison of the Effect of Bovine Platelet Lysate and Platelet Rich Plasma as Growth Promoters on Growth Rate and Viability of Different Cell Lines

Ahmad Erfanmanesh.[1*], Donya Nikaein.[2], Tahereh Mohajerfar.[1]

[1]*Academic Center for Education, Culture and Research (ACECR), Tehran Organization, Tehran, Iran*
[2]*Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Abstract:

BACKGROUND: Cell therapy and cell culture have received much attention in recent decades. Suitable cell growth requires growth supplements such as fetal bovine serum (FBS). FBS is component rich in nutrients, growth factors and supplementary compounds. However, FBS utilization has some limitations including mass production. Therefore, finding alternatives with the same growth promoting effects is inevitable.

OBJECTIVES: This study was designed to compare the effect of bovine platelet lysate (PL) and PRP on different cell lines as a cost effective and available alternative for FBS.

METHODS: Three conventional cell lines were investigated. Protein pattern of PL and platelet rich plasma (PRP) in comparison to FBS was determined using SDS page electrophoresis, and MTT and plating efficiency of cell lines in presence of PL and PRP were evaluated.

RESULTS: The results demonstrated that platelet rich plasma and platelet lysate could increase cells' viability similar to FBS. These results were significant in comparison with control group.

CONCLUSIONS: It can be concluded that platelet lysate could be a valuable candidate to replace FBS in cell culture techniques, however, more studies should be done to understand its exact efficacy.

Keywords:

Cattle, Fetal bovine serum, Fetal bovine serum alternatives, Platelet lysate, Platelet rich plasma

Correspondence

Ahmad Erfanmanesh.

Academic Center for Education, Culture and Research (ACECR), Tehran Organization, Tehran, Iran

Tel: +98(21) 66930415, Fax: +98(61) 66932999

Email: erfanmanesh@acecr.ac.ir

Received: 30 January 2018

Accepted: 6 May 2018

Introduction

Cell culture techniques have played an important role in cell biology and biomedical sciences in recent years (Abbott, 2003; McKee & Komarova, 2017; Witzeneder et al., 2013). Moreover, these techniques have replaced animal experiments in many clinical trials, however, production of high doses of cells is somewhat complicated (Abbott, 2003; Witzeneder et al., 2013). Growth supplements are necessary for natural metabolism, growth and mitogenicity of cells in culture conditions (Gershon, 1991). Fetal bovine serum (FBS) has been the most valuable supplementary product in cell culture techniques for many years (Bieback et al., 2009; Davey, Monte, Galel, Tressler, & Barlow, 2016; Gstraunthaler, 2003; Hemeda, Giebel, & Wagner, 2014; Kølle et al., 2013). According to previous reports production rate of FBS is about 500000 liters per year and this rate must increase each coming year (Brunner et al., 2010). However, FBS application has become challenging in recent years (Bieback et al., 2009; Rauch et al., 2011). It is mostly due to production limitations and variations in different batches of FBS. In addition, FBS might contain unwanted particles including endotoxins and xenogenic proteins (Rauch et al., 2011; Witzeneder et al., 2013). Hence researches have been done to find appropriate alternatives for FBS in cell culture and tissue engineering techniques (Bieback et al., 2009; Gstraunthaler, 2003; Kølle et al., 2013; McKee & Komarova, 2017; Rauch et al., 2011; Witzeneder et al., 2013).

Platelet rich plasma (PRP) is a blood derived component rich in growth factors and cytokines such as platelet-derived growth factor [PDGF] and transforming growth factor beta (TGFß), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (de Vos et al., 2010; Marx et al., 1998; Saucedo, Yaffe, Berschback, Hsu, & Kalainov, 2012; Schallmoser et al., 2007). It has been applied in bone and tissue healing and has gained interest in tissue and cell therapy (Marx, 2001). Platelet lysate (PL), derived from PRP, is rich in growth factors as well and it has been called a potential alternative to FBS in mesenchymal cell cultures (Capelli et al., 2007; Chevallier et al., 2010; Iudicone et al., 2014). However, there is still the risk of transmission of pathogens through blood collecting and providing high concentrations of human platelet lysate is difficult and costly (Iudicone et al., 2014). Thus, it is important to search for more cost effective and available products as an alternative to FBS.

This study was designed to investigate the growth promoting effects of bovine PRP and PL on three conventional cell lines in comparison with FBS as possible national produced candidates for FBS substitution in Iran.

Materials and Methods

This study was conducted in veterinary biological products research group of Academic center for Education, Culture and Research (ACECR), Tehran Organization.

Blood collection: Sterile blood was collected from Jugular vein of healthy calves, between 6-12 months old, kept in Mardabad Veterinary Research and Teaching Hospital, Faculty of Veterinary Medicine, University of Tehran. They were physically examined before blood collection by a veterinary internal medicine expert and their health was

confirmed. Sodium citrate 4% was used to avoid clot formation. Blood was transferred to laboratory under cool condition.

Preparation of PRP: PRP was obtained according to Akhundov et al method with minor modifications (Akhundov et al., 2012). In brief blood was centrifuged at 300g for 10 min. The supernatant containing platelets were collected and centrifuged for another 10 min in 1800g. The upper half of the supernatant was removed and cell pellet was resuspended in the remaining plasma.

Preparation of platelet lysate: Platelet lysate was prepared according to Schallmoser and Strunk method (Schallmoser & Strunk, 2009). First, PRP was prepared and kept at -20 °C. Platelets were lysed by putting frozen PRP for 10 min at room temperature and then two hours at 37 °C bath. Platelets were centrifuged at 4000 g, 4 °C for 15 min in order to remove cell particles. Supernatant was collected and sterile filtered using 0.22µ membrane filter. Heparin solution (0/002U/L, AlborzDarouCo Production, Iran) was added to prevent gel formation in culture condition.

Quality control (Sterility test): Microbial contamination was examined by culturing 100 µl of PRP and PL on blood agar and Sabouraud's dextrose agar (Merck, Germany). Samples were incubated at 37 °C. Any bacterial and/or fungal growth was investigated for seven days.

Evaluating the amount of platelets: Amount of platelets in PRP and PL were measured by hematology analyzer (Celltac, Nihonkohden, MEK-650) according to user instructions.

Protein measurement using Bradford method: Total protein in PRP and PL was measured using Bradford method. In brief absorption of each sample in presence of Bradford reagent was read at 595nm. Total protein was calculated from standard curve linear equation (Bradford, 1976).

Gel electrophoresis (SDS-PAGE): SDS-PAGE electrophoresis was used to compare protein patterns of PRP, PL and FBS. Resolving and stacking gels concentrations were 10% and 4% respectively. 15 µl of each diluted sample was added to SDS-PAGE wells. Protein marker covering molecular weights from 10-250 KDa was added to a single well, too. Electrophoresis was done on 80v voltage for 90min. Gel was fixed on glutaraldehyde 5% and dyed with Coomassie brilliant blue G for 20min. Gel was destained by acetic acid 10% for 3h (Su, Kuo, Tseng, Su, & Burnouf, 2009). Molecular weight and protein patterns of samples were determined comparing sample bonds with protein marker.

Growth promoting effect of PRP and PL on different cell lines (Cell line preparation): Three cell lines with different morphology characteristics including NIH/3T3 (fibroblastic), MDBK (epithelial) and Jurkat (lymphoblastoid) were purchased from Iranian Biological Research Center. Cells were cultured on high glucose DMEM medium containing 5% FBS and kept at 37 °C incubator with 5% CO2 before use.

MTT assay: This method was used to evaluate cells viability/proliferation (Coelho, Cabral, & Fernandes, 2000). Different Cell lines were trypsinized and adjusted to 106cells/ml. 100µl of cell suspension was added to each well of a 96 well plate. Then 100 µl of DMEM media containing concentration of 0, 5 and 10% of PRP, PL, and FBS in replicates of three was added to each well. Cells were incubated at 37 °C, 5% CO2 for 24h. At the end of 24h, plates were centrifuged and media was removed. 100 µl of

Table1. The comparison between different concentration of FBS, PRP and PL on viability of studied cell lines. Data are shown as mean ± SE. *different letters indicates significant difference (P<0.05). ** Capital letters indicate significant differences in 10% concentration of studied compounds, while small letters show significant differences in 5% concentrations.

Cell lines	FBS		Platelet lysate		PRP	
	5%	10%	5%	10%	5%	10%
NIH/3T3	145.87±7.80^{a}	160.24±6.58^{A}	164.22±12.71^{a}	225.08±4.28A**	149.54±14.83^{a}	205.81±34.56^{A}
MDBK	107.00±4.07^{b}	124.90±1.24^{B}	111.74±15.05^{b}	167.14±17.48^{B}	147.42±3.29^{a}	146.90±1.24^{B}
Jurkat	105.20±1.07^{b}	114.50±2.01^{B}	132.83±3.74^{c}	187.47±2.18^{C}	133.83±0.43^{b}	182.21±4.35^{C}

Table 2. Plating efficacy of NIH/3T3 and MDBK cell lines in presence of 5 and 10% FBS, PRP and PL. Data are shown as mean ± SE. *different letters indicates significant difference (P<0.05). **S=significant, NS=non significant. ***FBS: Fetal Bovine Serum, PRP: Platelet Rich Plasma, PL: Platelet Lysate.

Cell lines	FBS		PRP		PL		Control
	5%	10%	5%	10%	5%	10%	
NIH/3T3	16±0.0a	40±7.42bc	31.44±1,86b	70.78±16.70d	17.89±5.90a	53.8±30.08cd	0.44±0.88e
MDBK	16.33±11.92a	34.27±7.09b	13.17±1.33a	32.5±8.14b	31.33±5.55b	53.83±1.45c	0.5±0.57d
Significance	NS	NS	S	S	S	NS	-

MTT reagent (0.5mg/ml PBS) was poured to each well and cells were incubated at 37 °C, 5% CO2 for another 4 h. Then 100 µl isopropanol was added to wells to stop the reaction and well absorbance was read at 540nm. Below formula was used to determine viability of each cell line. All tests were done in three replicates.

Plating or cloning efficacy test: Different cell lines were cultured on DMEM medium and their number was adjusted to 3×103 cells /ml. The concentrations of 0, 5 and 10% of FBS, PRP and PL were added to each well of 6 well plates containing DMEM media in replicates of three. 0.1 ml of the prepared cell suspension (300 cells) was added into each well and plates were kept at 37 °C, 5% CO2 for eight days. After this time, culture media was removed and adhered cells were washed by PBS. Wells were stained with 1% methylene blue for 30 min and then the dye was removed and plates were washed with PBS. Number of colonies formed by each cell line was counted. Platting efficiency (PE) was calculated using the following formula:

PE (%) = (Colonies Counted / Cells Inoculated) x 100

(Taylor, Richter, Evans, & Sanford, 1974; Rheinwatd & Green, 1975)

Statistical analysis: Data were analyzed using SPSS software version 21. All numerical data are expressed as mean and standard deviation has been calculated. One way ANOVA and Dunnett's post hoc test were applied to investigate possible significant differences. Values of P<0.05 were considered significant.

Results

SDS-PAGE analysis and protein measurement: Total protein of PRP and PL was 52 and 50 mg/ml respectively. These amounts are equal to protein content of FBS. Figure 1 shows the protein pattern of PRP, PL and FBS on polyacrylamide gel. According to this figure there is only a slight difference between protein bonds of PRP/PL and FBS. This difference is in the 130Kd bond in which a bond is observed in PRP and PL electrophoresis.

MTT assay: Viability of three different cell lines, NIH/3T3, MDBK and Jurkat was

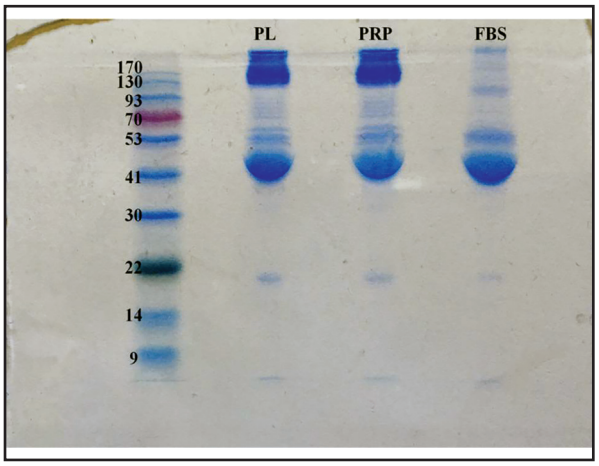

Figure 1. Protein patterns of bovine platelet lysate (PL), platelet rich plasma (PRP) and FBS on SDS-PAGE.

determined using MTT assay and 5 and 10% concentrations of FBS, PRP and PL. Viability percentage results are shown in Figs. 2, 3 and 4. In NIH/3T3 cell line PL and PRP in concentrations of 5 and 10% could increase the viability of cells equal to FBS in comparison to non-treated cell ($P<0.05$). Comparing the viability rates of NIH/3T3 cells in PRP and PL groups in comparison to FBS as control, cell viability had grown in PRP and PL group, however, this increase was not significant ($P>0.05$). PL had the highest effect on NIH/3T3 cells.

In MDBK cells, PL in the concentration of 5% could not significantly increase viability rate in comparison with the negative control group. All experimental groups had significantly increased the viability rate of cells in concentration of 10% ($P<0.05$). Putting FBS as the positive control, both PRP and PL groups had significantly higher viabilities ($P<0.05$); although in 5% concentration cells treated with PL had a viability equal to FBS ($P>0.05$). Interestingly, in 10% concentration PL had the highest viability rate among different groups while in 5% concentration PRP group hold the highest rank.

As for lymphoblastic cell line (Jurkat), in groups treated with 5% concentration of studied compositions, PRP and PL treated groups had the highest viability rates in comparison with the control group, however this difference was found significant only in 5% PRP concentration ($P<0.05$). Whilst in 10% concentrations, like MDBK cells the highest cell viability belonged to PL treated cells. The viability rate of cells treated with PRP and PL was significantly higher in the concentration of 10%, using FBS as the control for MTT calculations.

The effect of different concentrations of FBS, PRP and PL on all cell lines was studied as well. All compositions in concentrations of 5 and 10% had the highest growth promoting rate on NIH/3T3 cells ($P<0.05$). Whereas the lowest growth promoting rate belonged to MDBK cell line in all understudy compositions (Table 1).

Plating efficacy test: Table 2 shows plating efficacy results of FBS, PRP and PL on two under study cell lines. According to these results, in NIH/3T3 cell line, cloning efficacy had increased in all treatment groups ($P>0.05$). PRP at concentration of 5% showed highest colony formation ($P<0.05$). Although PL could induce more colonies in comparison to FBS, this difference was not significant ($P>0.05$).

In contrast, in MDBK cell line, PL induced most colonies in both concentrations ($P<0.05$) and although PRP treated group had more colony counts than FBS treated ones, no significant difference was observed between them ($P>0.05$). Since the appropriate method to study plating efficacy test on Jurkat cells could not be performed. This test was not applicable for this cell line.

Discussion

FBS has been used as a growth supple-

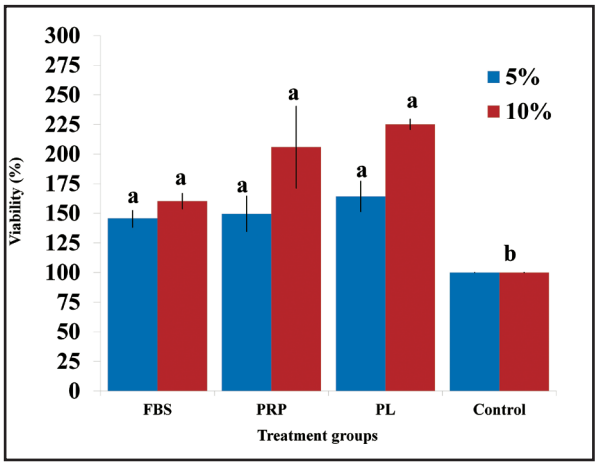

Figure 2. Viability percentage of NIH/3T3 cells treated with different concentration of fetal bovine serum (FBS), Platelet lysate (PL) and Platelet rich plasma (PRP) in comparison with non-treated control measured by MTT assay. *different letters indicates significant difference (P<0.05)

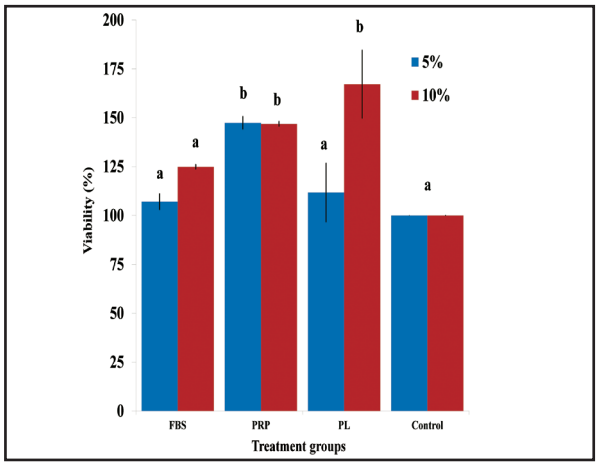

Figure 3.Viability percentage of MDBK cells treated with different concentration of fetal bovine serum (FBS), Platelet lysate (PL) and Platelet rich plasma (PRP) in comparison with non-treated control measured by MTT assay. *different letters indicates significant difference (P<0.05)

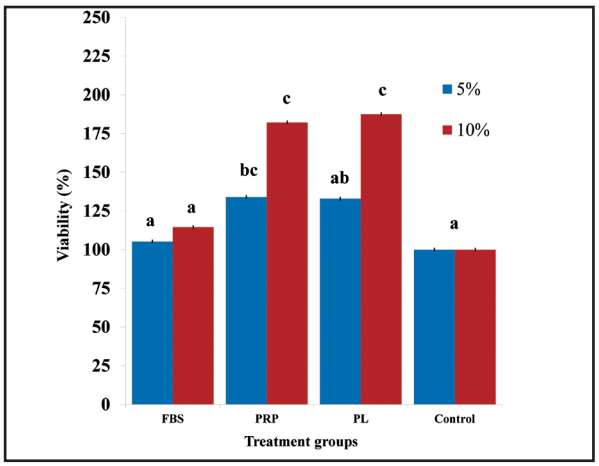

Figure 4.Viability percentage of Jurkat cells treated with different concentration of fetal bovine serum (FBS), Platelet lysate (PL) and Platelet rich plasma (PRP) in comparison with non-treated control by MTT assay. *different letters indicates significant difference (P<0.05)

ment in cell culture techniques for more than 50 years (Mannello & Tonti, 2007). However, development of cell culture application and limitations in FBS has increased the need for a suitable alternative to FBS (Gstraunthaler, Lindl, & van der Valk, 2013). Many researchers have studied the possible alternatives to FBS (Bieback et al., 2009; Gstraunthaler, 2003; Gstraunthaler et al., 2013; Rauch et al., 2011). Some of these products have been commercialized as well (Cao et al, 2009). But there is still the lack of a cost effective easy to use compound which is similar to FBS in composition and could be used in culture of almost every cell line and stem cell.

Platelets are known to have high levels of growth factors thus they could be an efficient replacement for FBS (Kølle et al., 2013; Marx, 2001; Marx et al., 1998). Human platelet lysate has been investigated as FBS alternative in mesenchymal cells and promising results have been obtained (Astori et al., 2016; Capelli et al., 2007; Schallmoser et al., 2007). However, a remarkable point in these studies is the lack of adequate blood donors in preparation of PL. It should be considered that expired platelets must be used with more caution because they could have gotten contaminated with pathogens and the derived lysate could be less efficient as well. By the way, Human PL availability is limited and it cannot be produced in large amounts. Our research studied calf Platelet lysate and PRP as potential alternatives to FBS; for this purpose composition and protein content of calf platelet lysate and PRP were compared to FBS and their effect on

proliferation and viability of the morphologically different cell lines using routine concentrations was investigated.

Our results demonstrated that protein content and composition of FBS, PL and PRP are very similar with a slight difference between 130 and 170 Kd region in which PRP and PL had an additional thick bond. This bond could be related to IgG, though further analysis is needed to identify the exact protein (Chou, Burnouf, & Wang, 2014). IgG might have negative results on therapeutic use of cell culture technique.

Studying the viability and proliferation of different cell lines treated with PL, PRP and FBS revealed that all treatment could significantly increase the viability of cell lines in comparison with the control group ($P<0.05$). No difference in gross morphology of cells was observed. Interestingly PL had the highest viability percentage while PRP usage showed most colony formations. The effect of PL on NIH/3T3 (fibroblast like) cells was better in comparison with other cell lines and this finding is inconsistent with other researches that studied the effect of human PL on mesenchymal cells (Capelli et al., 2007; Hemeda et al., 2014; Kølle et al., 2013; Schallmoser et al., 2007; Schallmoser & Strunk, 2009). Lower viability rates in MTT test of FBS treated cells could be because this product provides a long term promoting support for cells and thus a short term experiment alone cannot indicate FBS efficacies. In a study on equine platelet lysate, equine platelet lysate was added at concentrations of 5% to 60%, as an alternative to FBS, equine mesenchymal stromal cells (MSC). No significant difference was found between PL and FBS cultured cells in concentrations up to 30%, however cell numbers reduced in higher PL concentrations. The authors concluded that equine PL is efficient for short term proliferation of MSC (Russell & Koch, 2016). However, our results on plating efficiency of different cell lines demonstrate that PL and PRP are suitable for long term proliferation of cells as well.

Huang et al. investigated the replacement of FBS with plasma derived from human umbilical cord blood (HCP) on endothelial colony forming cells (ECFC). These cells are very dependent on FBS for proliferation. They could successfully isolate and proliferate human ECFC in vitro using a low concentration of HCP (1.5%) and no FBS supplementation (Huang, Critser, Grimes, & Yoder, 2011). Since human umbilical blood is not available in large scale, it does not seem to be an appropriate replacement for FBS. Considering this limitation, calf serum and platelet lysate are more applicable.

In a study in 2014 in Iran, the effect of human PRP as an alternative to FBS was investigated in the proliferation and differentiation of mesenchymal stem cells. The results of this study showed a significant increase in the proliferation of mesenchymal stem cells in PRP treated cells (Khosravi, Mossavi, Parizadeh, & Aghagolzade Haji, 2014). Similar results were seen in our study as well and PRP was more efficient in colony forming of NIH/3T3 and MDBK cells than FBS.

In conclusion, our study illustrated the proper effects of calf derived PRP and PL on proliferation and colony formation of three cell lines. According to our results PL is more suitable alternative to FBS. Although more studies are needed to clarify safety of PL applications in cell culture techniques, its shelf life, and its exact composition. In addition, since FBS must be replaced with a

product which is more accessible and easier to be generated in high volumes, researches must be designed to determine the best method for high volume production.

Acknowledgements

This study was supported by the research and technology deputy of Academic Center for Education, Culture and Research (ACECR), Iran.

References

Abbott, A. (2003) Cell culture: biology's new dimension. Nature. 424(6951): 870-872.

Akhundov, K., Pietramaggiori, G., Waselle, S. D., Guerid, S., Scaletta, C., Hirt-Burri, N., Applegate, L.A., Raffoul, W. (2012) Development of a cost-effective method for platelet-rich plasma (PRP) preparation for topical wound healing. Ann Burns Fire Disasters. 25(4): 207.

Astori, G., Amati, E., Bambi, F., Bernardi, M., Chieregato, K., Schäfer, R., Sabrina, S., Rodeghiero, F. (2016) Platelet lysate as a substitute for animal serum for the ex-vivo expansion of mesenchymal stem/stromal cells: present and future. Stem Cell Res Ther. 7(1): 93.

Bieback, K., Hecker, A., Kocaömer, A., Lannert, H., Schallmoser, K., Strunk, D., & Klüter, H. (2009) Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. Stem Cells. 27(9): 2331-2341.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72(1-2): 248-254.

Brunner, D., Frank, J., Appl, H., Schöffl, H., Pfaller, W., Gstraunthaler, G. (2010) Serum-free cell culture: the serum-free media interactive online database. Altex. 27(1): 53.

Capelli, C., Domenghini, M., Borleri, G., Bellavita, P., Poma, R., Carobbio, A., Mico, C., Rambaldi, A., Golay, J., Introna, M. (2007) Human platelet lysate allows expansion and clinical grade production of mesenchymal stromal cells from small samples of bone marrow aspirates or marrow filter washouts. Bone Marrow Transplant. 40(8): 785-791.

Cao, Z., West, C., Norton-Wenzel, C. S., Rej, R., Davis, F. B., Davis, P. J., Rej, R. (2009) Effects of resin or charcoal treatment on fetal bovine serum and bovine calf serum. Endocrine Research. 34(4): 101-108.

Chevallier, N., Anagnostou, F., Zilber, S., Bodivit, G., Maurin, S., Barrault, A., Bierling, Ph., Hernigou, Ph., Layrolle, P., Rouard, H. (2010) Osteoblastic differentiation of human mesenchymal stem cells with platelet lysate. Biomaterials. 31(2): 270-278.

Chou, M.-L., Burnouf, T., Wang, T.-J. (2014) Ex vivo expansion of bovine corneal endothelial cells in xeno-free medium supplemented with platelet releasate. PloS one. 9(6): e99145.

Coelho, M., Cabral, A. T., Fernandes, M. (2000) Human bone cell cultures in biocompatibility testing. Part I: osteoblastic differentiation of serially passaged human bone marrow cells cultured in α-MEM and in DMEM. Biomaterials. 21(11): 1087-1094.

de Vos, R. J., Weir, A., van Schie, H. T., Bierma-Zeinstra, S. M., Verhaar, J. A., Weinans, H., Tol, J. L. (2010) Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA. 303(2): 144-149.

Gershon, D. (1991) Cell culture supplement. Nature. 352: 829-832.

Gstraunthaler, G. (2003) Alternatives to the use of fetal bovine serum: serum-free cell culture. Altex. 20(4): 275-281.

Gstraunthaler, G., Lindl, T., van der Valk, J. (2013) A plea to reduce or replace fetal bovine serum in cell culture media. Cytotechnology. 65(5): 791-793.

Hemeda, H., Giebel, B., Wagner, W. (2014) Evaluation of human platelet lysate versus fetal bovine serum for culture of mesenchymal stromal cells. Cytotherapy. 16(2): 170-180.

Hemeda, H., Giebel, B., Wagner, W. (2014) Evaluation of human platelet lysate versus fetal bovine serum for culture of mesenchymal stromal cells. Cytotherapy. 16(2): 170-180.

Huang, L., Critser, P. J., Grimes, B. R., Yoder, M. C. (2011) Human umbilical cord blood plasma can replace fetal bovine serum for in vitro expansion of functional human endothelial colony-forming cells. Cytotherapy. 13(6): 712-721.

Iudicone, P., Fioravanti, D., Bonanno, G., Miceli, M., Lavorino, C., Totta, P., Frati, L., Nuti, M., Pierelli, L. (2014) Pathogen-free, plasma-poor platelet lysate and expansion of human mesenchymal stem cells. Journal of translational medicine. 12(1): 28.

Khosravi, M., Mossavi, N. S., Parizadeh, S. M. R., Aghagolzade Haji, H. (2014) Investigating platelet rich plasma effects on adipose-derived stromal cellsproliferation and differentiation on osteoblast. Urmia Medical Journal. 25(5): 453-462.

Kølle, S.-F. T., Oliveri, R. S., Glovinski, P. V., Kirchhoff, M., Mathiasen, A. B., Elberg, J. J., Anderson, P. S., Drzewiecki K. T., Fischer-Nielsen, A. (2013) Pooled human platelet lysate versus fetal bovine serum—investigating the proliferation rate, chromosome stability and angiogenic potential of human adipose tissue-derived stem cells intended for clinical use. Cytotherapy. 15(9): 1086-1097.

Listing, T. S. H. S. P. Thermo Scientific HyClone Media and Sera Table of Contents.

Mannello, F., Tonti, G. A. (2007) Concise Review: No Breakthroughs for Human Mesenchymal and Embryonic Stem Cell Culture: Conditioned Medium, Feeder Layer, or Feeder-Free; Medium with Fetal Calf Serum, Human Serum, or Enriched Plasma; Serum-Free, Serum Replacement Nonconditioned Medium, or Ad Hoc Formula? All That Glitters Is Not Gold! Stem Cells. 25(7): 1603-1609.

Marx, R. E. (2001) Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 10(4): 225-228.

Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E., Georgeff, K. R. (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiol Endodontol. 85(6): 638-646.

McKee, T. J., Komarova, S. V. (2017) Is it time to reinvent basic cell culture medium? Am Physiological Soc. (Vol. 312, pp. 624-626).

Rauch, C., Feifel, E., Amann, E.-M., Peter Spötl, H., Schennach, H., Pfaller, W., Gstraunthaler, G. (2011) Alternatives to the use of fetal bovine serum: human platelet lysates as a serum substitute in cell culture media. ALTEX-Alternatives to Animal Experimentation. 28(4): 305.

Rheinwatd, J. G., Green, H. (1975) Seria cultivation of strains of human epidemal keratinocytes: the formation keratinizin colonies from single cell is. Cell. 6(3): 331-343.

Russell, K., Koch, T. (2016) Equine platelet lysate as an alternative to fetal bovine serum in equine mesenchymal stromal cell culture–too much of a good thing? Equine Vet J. 48(2): 261-264.

Saucedo, J. M., Yaffe, M. A., Berschback, J. C., Hsu, W. K., Kalainov, D. M. (2012) Platelet-rich plasma. J Hand Surg. 37(3): 587-589.

Schallmoser, K., Bartmann, C., Rohde, E., Reinisch, A., Kashofer, K., Stadelmeyer, E., Drex-

ler, C., Lanzer, G., Linkesch, W., Strunk, D. (2007) Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells. Transfusion. 47(8): 1436-1446.

Schallmoser, K., Strunk, D. (2009) Preparation of pooled human platelet lysate (pHPL) as an efficient supplement for animal serum-free human stem cell cultures. J Vis Exp. JoVE(32).

Su, C. Y., Kuo, Y. P., Tseng, Y. H., Su, C.-H., Burnouf, T. (2009) In vitro release of growth factors from platelet-rich fibrin (PRF): a proposal to optimize the clinical applications of PRF. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiol Endodontol. 108(1): 56-61.

Taylor, W. G., Richter, A., Evans, V. J., Sanford, K. K. (1974) Influence of oxygen and pH on plating efficiency and colony development of WI-38 and Vero cells. Exp Cell Res. 86(1): 152-156.

Witzeneder, K., Lindenmair, A., Gabriel, C., Höller, K., Theiß, D., Redl, H., Hennerbichler, S. (2013) Human-derived alternatives to fetal bovine serum in cell culture. Transfus Med Hemother. 40(6): 417-423.

مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۳، ۲۵۸-۲۴۹

مقایسه اثر لیزات پلاکتی و پلاسمای غنی از پلاکت گوساله به عنوان محرک رشد بر میزان رشد و قابلیت حیات رده‌های مختلف سلولی

احمدعرفان‌منش[1*] دنیا نیک‌آئین[2] طاهره مهاجرفر[1]

۱) گروه پژوهشی فرآورده‌های بیولوژیک دامی، سازمان جهاددانشگاهی تهران جهاددانشگاهی، تهران ایران
۲) مرکز تحقیقات قارچ‌شناسی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

(دریافت مقاله: ۱۰ بهمن ماه ۱۳۹۶، پذیرش نهایی: ۱۶ فروردین ماه ۱۳۹۷)

چکیده

زمینه مطالعه: سلول‌درمانی و کشت سلولی در دهه‌های اخیر توجه زیادی را به خود جلب کرده است. رشد مناسب سلول‌ها در محیط کشت نیازمند استفاده از مکمل‌های رشد مانند سرم جنین گاو (FBS) است. ترکیبFBS غنی از مواد مغذی، فاکتورهای رشد و ترکیبات مکمل است. با این وجود استفاده از FBS محدودیت هایی دارد که از آن جمله تولید انبوه آن می‌باشد. بنابراین پیدا کردن جایگزینی با همان اثرات محرک رشد اجتناب ناپذیر است.

هدف: این مطالعه به منظور مقایسه اثر لیزات پلاکتی گاو(PL) و پلاسمای غنی از پلاکت (PRP) بر روی رده‌های مختلف سلولی به عنوان یک روش ارزان و موثر برای جایگزینی FBS طراحی شده است.

روش کار: در این مطالعه سه رده‌ی سلولی متعارف مورد بررسی قرار گرفتند. الگوی پروتئین PL و PRP در مقایسه با FBS با استفاده از الکتروفورز SDS PAGE تعیین شد و MTT و قابلیت تشکیل کلونی در حضور PL و PRP به دست آمد.

نتایج: نتایج نشان داد که پلاسمای غنی از پلاکت و لیزات پلاکتی می‌توانند حیات سلولی را مشابه با FBS افزایش دهند. این نتایج در مقایسه با گروه شاهد معنی‌دار بود.

نتیجه‌گیری نهایی: بنابراین می‌توان نتیجه گرفت که لیزات پلاکتی گوساله می‌تواند یک جایگزین مطرح برای FBS در تکنیک‌های کشت سلولی باشد اما مطالعات بیشتری باید انجام شود تا بتواند اثر دقیق آن را درک کند.

واژه‌های کلیدی: گوساله، سرم جنین گوساله، جایگزین سرم جنین، لیزات پلاکتی، پلاسمای غنی از پلاکت

---

*) نویسنده مسؤول: تلفن: ۶۶۹۳۰۴۱۵ (۲۱)۹۸+ نمابر: ۶۶۹۳۲۹۹۹ (۲۱)۹۸+ Email: erfanmanesh@acecr.ac.ir