

HUMAN PLASMA AND HUMAN PLATELET-RICH PLASMA AS A SUBSTITUTE FOR FETAL CALF SERUM DURING LONG-TERM CULTIVATION OF MESENCHYMAL DENTAL PULP STEM CELLS

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Summary: Aims: Our aims were to isolate and cultivate mesenchymal dental pulp stem cells (DPSC) in various media enriched with human blood components, and subsequently to investigate their basic biological properties. Methods: DPSC were cultivated in five different media based on α MEM containing different concentrations of human plasma (HP), platelet-rich plasma (PRP), or fetal calf serum (FCS). The DPSC biological properties were examined periodically. Results: We cultivated DPSC in the various cultivation media over 15 population doublings except for the medium supplemented with 10% HP. Our results showed that DPSC cultivated in medium supplemented with 10% PRP showed the shortest average population doubling time (DT) (28.6 ± 4.6 hours), in contrast to DPSC cultivated in 10% HP which indicated the longest DT (156.2 ± 17.8 hours); hence this part of the experiment had been cancelled in the 6th passage. DPSC cultivated in media with 2% FCS+ITS (DT 47.3 ± 10.4 hours), 2% PRP (DT 40.1 ± 5.7 hours) and 2% HP (DT 49.0 ± 15.2 hours) showed almost the same proliferative activity. DPSC's viability in the 9th passage was over 90% except for the DPSC cultivated in the 10% HP media. Conclusions: We proved that human blood components are suitable substitution for FCS in cultivation media for long-term DPSC cultivation.

Key words: Dental pulp stem cells; Stem cells; Cultivation medium; Fetal calf serum; Human plasma; Platelet-rich plasma; Long-term cultivation

Introduction

Stem cells (SC) constitute one of the first cell populations in the nascent organism. Their main roles are to produce specialized cell types and simultaneously to maintain the SC population into adulthood. For this reason, SC are endowed with special properties that distinguish them from all the other cells in the body; such as high proliferative activity, differentiation potential, and the ability of self-renewal. Altogether SC play the key role in development and regeneration of damaged tissues. The growing body of knowledge about their behavior and properties suggests that SC have a great potential as a tool for tissue engineering and cell therapy.

Dental pulp stem cells (DPSC) are typically isolated by enzymatic dissociation of dental pulp tissue, obtained most often from non-erupted lower third molars. They exhibit typical fibroblast-like morphology and retain high proliferation ability, even during prolonged cultivation (1). DPSC express the cell surface mesenchymal stem cells markers and also some markers of bone marrow stem cells (STRO-1 and CD-146) as well as embryonic stem cells markers

(Oct4) (2). DPSC cultivation in osteogenic, chondrogenic, dentinogenic, adipogenic, neurogenic and myogenic medium demonstrated their wide differential potential (2–4). Dental pulp is easily accessible and ethically acceptable source of SC.

Fetal calf serum (FCS, FBS) became, due to its high content of growth factors and conversely a low content of inhibitory factors, the most widely used growth supplement in cultivation media. Due to the origin of the serum, it is not possible to maintain stable and unchanging composition of individual batches (different amount of protein, other bioactive substances, endocrine parameters etc.) (5, 6). Many current clinical trials use cultivation media for stem cells enriched with FCS, even though FCS is a potentially dangerous xenogeneic component. Particularly bovine serum proteins can be internalized by stem cells and stimulate immunogenicity (7, 8) in this context many problems may also arise due to viral and prion transmission (9, 10), or immunological reactions as responses to antigenic substrate (11). Although these “side effects” are well realized, in literature they are often overlooked; an example would be a treatment of myocardial infarction by myoblasts cultured

in FCS, which resulted in the formation of malignant ventricular arrhythmias and death of the patient (12); treatment or lack of adenosine deaminase with autologous T cells cultured in medium with FCS supplemented leading to the development of IgG antibodies against FCS (13). Bovine serum contains many polypeptides which can induce metabolic and morphological changes (14, 15). Therefore, the interest of the scientific community turns to human blood components or serum-free media containing artificial supplements (16, 17). Although, it isn't possible to standardize the human blood components, their usage avoids internalization of the animal substances, thus excluding the risk of zoonosis and immunogenicity (7, 9, 10). As the cultivation supplement we can use manufactory produced approved AB-serum or umbilical cord blood, which is very hard to obtain. Stojkovic proved that embryonic stem cells cultivated in medium with human serum retain all the characteristics of embryonic cells during long-term cultivation and have unlimited proliferation capacity while maintaining a stable karyotype (18) and Bo Chen, Ui-Lyong Lee and Vijayendram (19, 20, 21) proved that DPSC can be cultivated in presence of PRP without losing their SC characteristics and differentiation capacity. The effect of platelet rich plasma (PRP) and plasma (HP) derived from human blood is based on degranulation α granules from activated platelets, which contain growth factors. These growth factors are platelet derived growth factor (PDGF), transforming growth factors beta 1 and beta 2 (TGF β 1, TGF β 2), vascular endothelial growth factor (VEGF), platelet derived endothelial cell growth factor, interleukin-1 (IL-1), basic fibroblast growth factor (bFGF), and platelet activating factor-4 (PAF-4) (22, 23). The human blood serum is obtained by centrifugation of clotted blood (24). Platelet concentrates are defined as components which have 3 to 5 fold higher concentration of platelets in the plasma compared to whole blood (25). It was proved that stem cells cultivated in a media enriched with PRP maintain differentiation capacity, demonstrate higher proliferative activity (19–21, 26–30), while it was also shown that human serum alone does not fully support the growth of human mesenchymal stem cells in vitro (31). Another option for replacing the FCS is artificially mixed supplement, but due to the complexity and not completely known composition of FCS it will be very expensive and hard to obtain it.

The aim of this study is to determine the effect of different human blood components on cultivated dental pulp stem cells and if these supplements could be used instead of FCS.

Material and Methods

The dental pulp (DP) used in the experiment was obtained from the twenty years old male patient, who undergone extraction of an impacted third molar due to an orthodontic indication. Prior to donation the patient was informed of the stem cell theory, the planned experiment and has signed an informed consent. The isolation of the DPSC for the experi-

mental purposes, the informed consent and a text containing information for the patient were approved by the Ethical Committee of the University Hospital Hradec Králové ref. 200712 S01P; the obtaining and use of blood products in the culture medium was also approved by the Ethical Committee of the University Hospital Hradec Králové ref. 201011 S14P. Blood components were prepared in collaboration with the Transfusion Department.

Tooth extraction was performed under standard conditions under local anesthesia. The tooth has been treated with disinfectant and transported in a closed container completely immersed in a transport medium composed of 1 ml of HBSS (Invitrogen, USA), 9 ml water for inj. (Bieffe Medital, Italy), 200 μ l/10 ml gentamycin (Invitrogen, USA), 200 μ l/10 ml streptomycin (Invitrogen, USA), 200 μ l/10 ml amphotericin (Sigma, USA) and 200 μ l/10 ml penicillin (Invitrogen, USA) at 4 °C.

The isolation of the DP and the DPSC from the tooth took place under sterile conditions in a tissue culture laboratory at the Department of Histology and Embryology LFHK by enzymatic dissociation (DP was cut into pieces of about 1 mm³, enzymatically dissociated for 50 minutes at temperature of 37.0 °C in a solution of enzyme collagenase type I (Sevapharma, CR), dispase (Invitrogen, USA), PBS (Invitrogen, USA) and HBSS (Invitrogen, USA) in the 1:1:1:1 ratio). The obtained cell suspension was centrifuged for 5 minutes at 2000 rev/min (600 g). The resulting cell pellet was resuspended and inoculated into culture vessels, Cell + (Sarstedt, USA).

DPSC were cultured under standard conditions in an incubator at 37 °C, in an atmosphere with 5% CO₂ in the culture medium consisting of α -MEM (Invitrogen, USA), 2% FCS (PAA, USA), 10 ng/ml EGF (PeproTech, USA), 10 ng/ml PDGF (PeproTech, USA), L-ascorbic acid (Sigma, USA), 2% glutamine (Invitrogen, USA), penicillin/streptomycin (Invitrogen, USA), gentamycin (Invitrogen, USA), dexamethasone (Sigma, USA) and supplemented with insulin-transferrin-sodium-selenite supplement (ITS) (Sigma, USA) 10 μ l/ml. DPSC obtained from 3rd passage were frozen and stored. After the DPSC bypassed the Hayflick limit; the DPSC ability to differentiate in osteoblast-like cells was verified. The cells obtained from the third passage were thawed and seeded into 5 different media (Tab. 1).

Medium 1 (standard medium): α -MEM (Invitrogen, USA), 2% FCS (PAA, USA), 10 ng/ml EGF (PeproTech, USA), 10 ng/ml PDGF (PeproTech, USA), L-ascorbic acid (Sigma, USA), 2% glutamine (Invitrogen, USA), penicillin/streptomycin (Invitrogen, USA), gentamycin (Invitrogen, USA), dexamethasone (Sigma, USA) and 10 μ l of ITS/ml (Sigma, USA).

Medium 2: α -MEM (Invitrogen, USA), 2% HP, 10 ng/ml EGF (PeproTech, USA), 10 ng/ml PDGF (PeproTech, USA), L-ascorbic acid (Sigma, USA), 2% glutamine (Invitrogen, USA), penicillin/streptomycin (Invitrogen, USA), gentamycin (Invitrogen, USA), dexamethasone (Sigma, USA) and 10 μ l of ITS/ml (Sigma, USA).

Medium 3: α -MEM (Invitrogen, USA), 10% HP, L-ascorbic acid (Sigma, USA), 2% glutamine (Invitrogen, USA), penicillin/streptomycin (Invitrogen, USA), gentamycin (Invitrogen, USA), dexamethasone (Sigma, USA).

Medium 4: α -MEM (Invitrogen, USA), 2% PRP, 10 ng/ml EGF (PeproTech, USA), 10 ng/ml PDGF (PeproTech, USA), L-ascorbic acid (Sigma, USA), 2% glutamine (Invitrogen, USA), penicillin/streptomycin (Invitrogen, USA), gentamycin (Invitrogen, USA), dexamethasone (Sigma, USA) and 10 μ l of ITS/ml (Sigma, USA).

Medium 5: α -MEM (Invitrogen, USA), 10% PRP, L-ascorbic acid (Sigma, USA), 2% glutamine (Invitrogen, USA), penicillin/streptomycin (Invitrogen, USA), gentamycin (Invitrogen, USA), dexamethasone (Sigma, USA).

The experiment used pooled HP and PRP obtained from 5 donors according to the protocols of Transfusion Department FNHK.

The basic biological characteristics were measured during the cultivations. Proliferative activity and population doubling time were measured for every passage using Z2

Counter (Beckman Coulter, USA). Viability was measured using Vi-Cell analyzer (Beckman Coulter, USA). Phenotypic analysis was performed using Cell Lab Quanta (Beckman Coulter, USA), using the following antibodies: CD29 (BD Biosciences Pharmingen, Belgium), CD44 (BD Biosciences Pharmingen, Belgium), CD73 (BD Biosciences Pharmingen, Belgium), CD90 (BD Biosciences Pharmingen, Belgium), CD105 (Invitrogen, California, USA) and CD 146 (BD Biosciences Pharmingen, Belgium).

Statistical Analysis

Data are presented as arithmetic mean \pm SD. All statistical tests were performed using Microsoft Office Excel 2010 (Microsoft, Redmond, WA).

Results

The DPSC were spindle-shaped with elongated processes. DPSC cultivated in media 1 were more rounded

Tab. 1: Composition of the test media and the quantities of supplements per 100 ml of media.

MEDIUM/ SUPPLEMENT	1	2	3	4	5
α-MEM	94.96 ml	94.96 ml	86 ml	94.96 ml	86 ml
(Invitrogen, USA)					
L-ascorbic acid	1 ml	1 ml	1 ml	1 ml	1 ml
(Sigma, USA)	(10 mg)	(10 mg)	(10mg)	(10 mg)	(10 mg)
Glutamin	1.9 ml	1.9 ml	1.9 ml	1.9 ml	1.9 ml
(Invitrogen, USA)					
PEN/STM	0.6 ml	0.6 ml	0.6 ml	0.6 ml	0.6 ml
(Invitrogen, USA)					
GEN	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
(Invitrogen, USA)					
Dexametazone	8 μ l/ml	8 μ l/ml	–	8 μ l/ml	–
(Sigma, USA)					
PDGF-BB	10 ng/ml	10 ng/ml	–	10 ng/ml	–
(Peprotech, USA)					
EGF	10 ng/ml	10 ng/ml	–	10 ng/ml	–
(Peprotech, USA)					
ITS	10 μ l/ml	10 μ l/ml	–	10 μ l/ml	–
(Sigma, USA)					
FCS	1.94 ml	–	–	–	–
(PAA, USA)	2%				
HP	–	1.94 ml	9.7 ml	–	–
		2%	10%		
PRP	–	–	–	1.94 ml	9.7 ml
				2%	10%

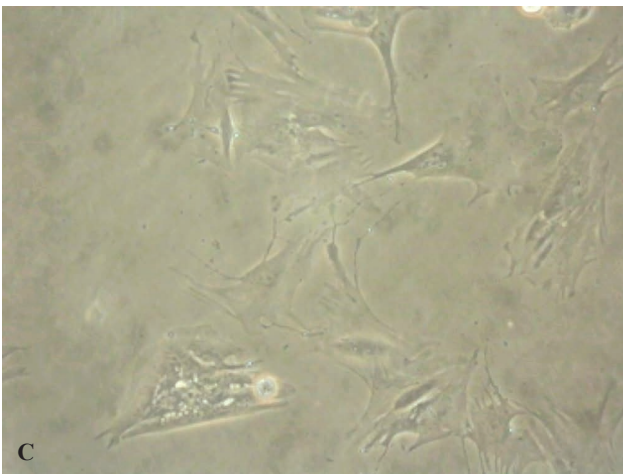
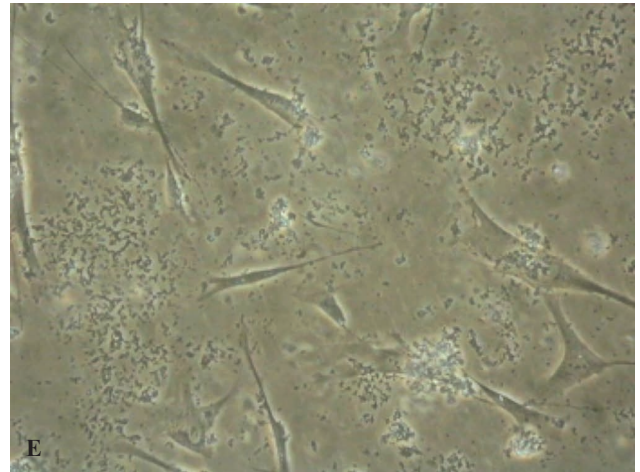
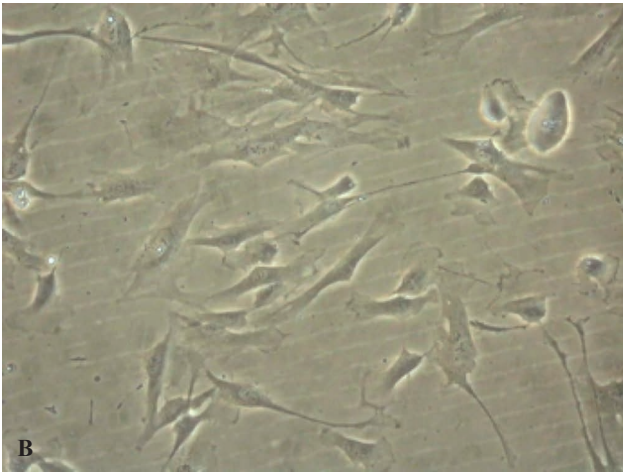
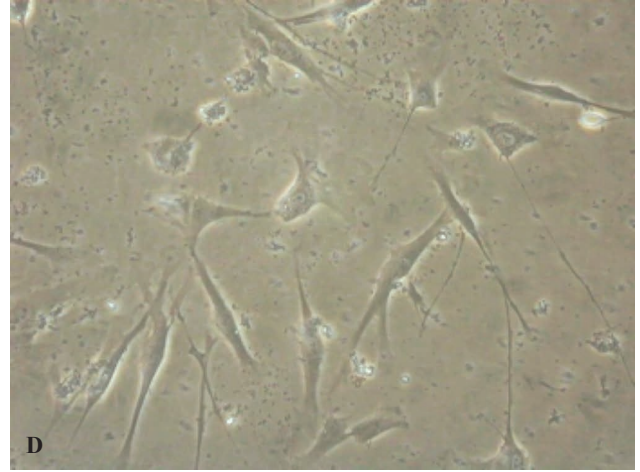
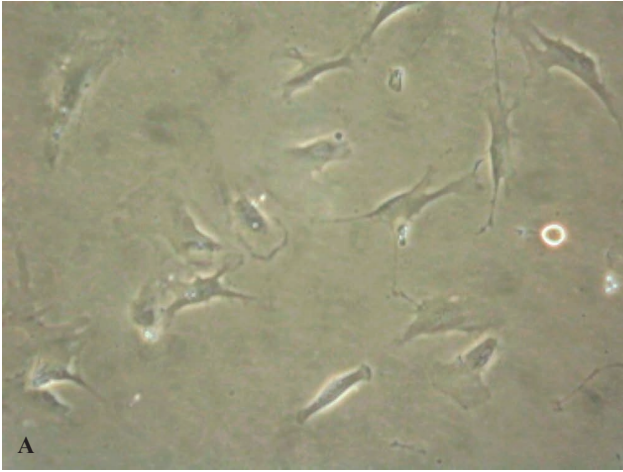
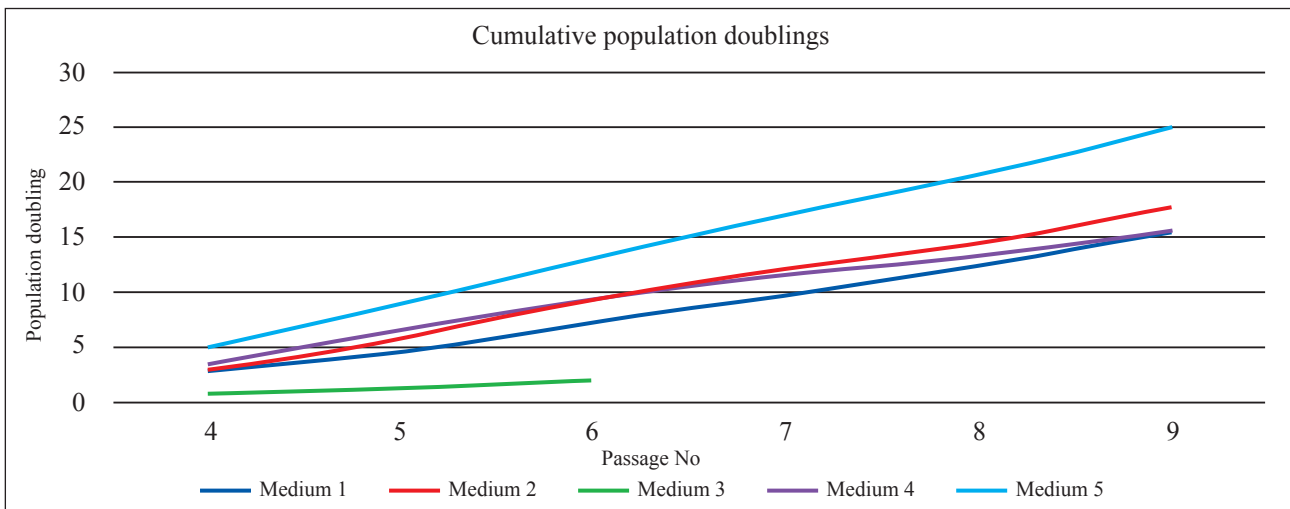


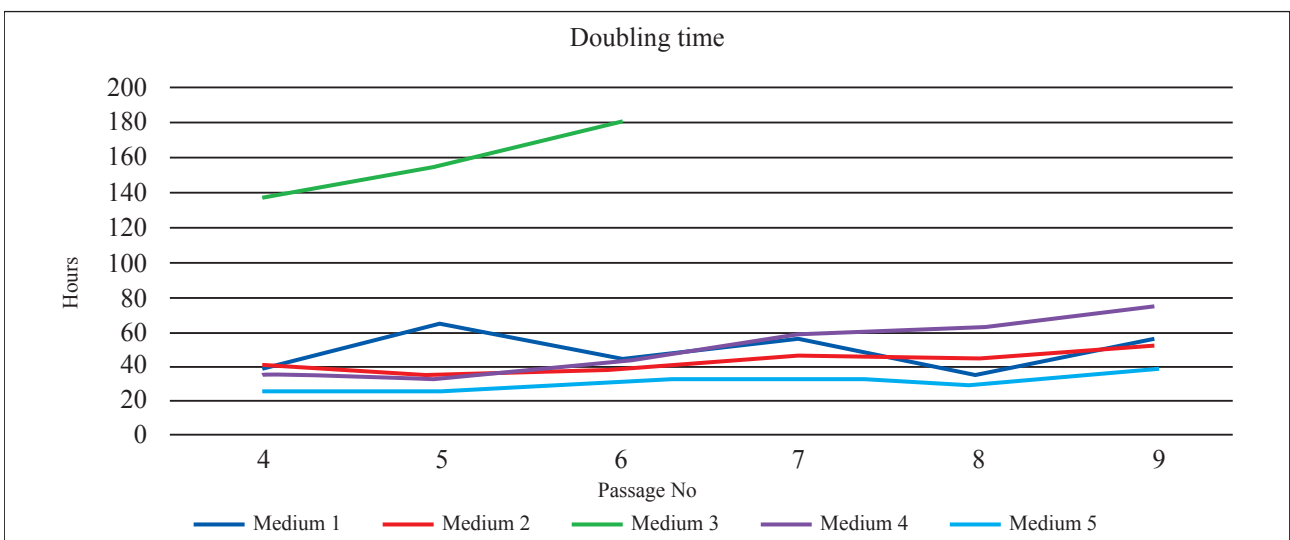
Fig. 1: A) Stem cells cultured in the media 1, the image was captured in the 9th passage, 3 days after seeding into the culture vessel. B) Stem cells cultured in the media 2, the picture taken in the 9th passage, 2 days after seeding into the culture vessel. C) Stem cells cultured in the media 3, the picture was taken in the 5th passage 3 days after seeding into the culture vessel. DPSC were more adherent to the surface of the culture vessel than in other media, therefore they seem to be bigger. D) Stem cells cultured in the media 4, the picture taken in the 9th passage, 3 days after seeding into the culture vessel. E) Stem cells cultured in the media 5, the picture was taken in the 9th passage, 3 days after deployment into the culture vessel. In the Figures A, B, D, E we can see the typical DPSC morphology. In the Figures D, and E, we can see tiny particles that float freely in the culture media. A–E pictures were taken with a phase contrast microscope, original magnification 200 \times .

in compare to those cultivated in media 2, 4, 5. DPSC cultivated in media 2 and 4 share similar morphology and those cultivated in media 5 were more prolonged and less adhered. In media 3 the DPSC were more adhered to the surface of the culture vessel. All the tested media with PRP (2%, 10%) contained microscopic inhomogeneous particles. These were most probably the aggregated residues of the platelets membranes. We assume so because these particles were not found in any other tested media and the presence of platelets was the only difference; moreover in the media 5 (10% PRP) we found substantially more of these (Fig. 1, A–E). DPSC cultured in media 5 exhibited the highest proliferative activity and the associated the

shortest population doubling time. Total population doubling achieved in this media was 25.3, the average doubling time was 28.6 ± 4.6 hours. The slowest proliferative activity was achieved in the third media. Total population doubling achieved in this media throughout the experiment was 2.18, the average doubling time was 156.2 ± 17.8 hours. The cells in this media essentially did not proliferate and testing of the third media was stopped at the 6th passage. DPSC cultured in media 1, 2 and 4 exhibited very similar proliferative activity. Total population doublings reached in these media (1, 2, 4 media order) were 15.78, 18.0 and 15.7, the average doubling times were 47.3 ± 10.4 , 40.1 ± 5.7 and 49.0 ± 15.2 hours (Graph 1, 2).



Graph 1: Cumulative number of the population doubling in the various test media. Test media 3 was stopped in the 6th passage for a very low number the DPSC. Total population doubling achieved in the media, throughout the experiment, ordered by media numbers: 1, 2, 3, 4, and 5 were 15.78, 18.0, 2.18, 15.7, and 25.3.



Graph 2: Doubling times during the experiment, ordered by media numbers: 1, 2, 3, 4, and 5 – the average doubling times were 47.3 ± 10.4 , 40.1 ± 5.7 , 156.2 ± 17.8 , 49.0 ± 15.2 , and 28.6 ± 4.6 hours. Testing medium 3 was stopped in the 6th passage for a very low number the DPSC.

DPSC cultivated in all media showed a very high viability, usually in excess of 90%. The viability of DPSC was measured in the 9th passage. Only in the media 3, we were forced to measure the viability already in the 6th passage because of the low proliferative activity and termination of the testing media. Viability in the media 1 was 96.2%, 96.6% in the media 2, 88.1% in the media 3, 96.3% in the media 4, and 91.7% in the media 5.

DPSC cultured in the media 2 showed, compared to the control group cultured in the standard media with FCS, the same expression of tested markers CD 29, CD 73, CD 105 and CD 146, while the positivity for CD 44 increased to 64.78% compared to 41.7%. The DPSC cultivated in the media 4 and 5 showed the same positivity for the CD 29, but compared to standard media significantly lower positivity for other tested markers. Due to the low proliferative activity it was impossible to do the phenotypic analysis of the DPSC cultured in the media 3. The positivity for each marker in media order 1,2,4,5 were for CD 29 – 86.1% / 81.09% / 89.49% / 98.56%, for CD 44 – 41.7% / 64.78% / 33.26% / 10.66%, for CD 73 – 67.81% / 73.62% / 34.94% / 12.77%, for CD 90 – 82.15% / 85.49% / 49.24% / 12.05%, for CD 105 – 26.92% / 38.28% / 10.2% / 5.12%, for CD 146 – 63.94% / 68.66% / 40.45% / 41.72% (Table 2).

Discussion

Despite the stem cells research is already quite advanced, the usage of cell therapy (32, 33, 34) is not possible in every day human medicine. One of the major reason is the usage of xenogeneic supplements during the SC cultivation (35).

Even though the xenogeneic components represent cheap and easily accessible source of growth factors and substances important for the stem cells proliferation, their usage can cause internalisation of xenogeneic proteins into the cell membrane and transmission of zoonoses, viral or prion infection. The internalized xenogeneic proteins, which are arduous to detect, can later lead to immunological reaction in patients (36, 37).

In our study we compared 2 different human blood components in different concentrations and successfully re-

placed the FCS in cultivation media. To obtain comparable data we decided to test all 5 different media on one lineage. We are fully aware that DPSC were cultivated in a medium containing 2% FCS during first three passages and this may affect the results of the experiment, but on the other hand through this procedure we have been able to clearly define and demonstrate the changes in phenotype, proliferation ability and viability of the tested DPSC. To investigate the differences among the properties of hMSC cultivated in various media supplemented with FCS, or human blood components (49, 50, 51, 52, 19, 20, 21); however neither of them provides statistically relevant results on which we could build.

The hMSC cultured in media of different compositions resulted in similar morphological variances as previously described for hMSC (38, 42, 19, 20, 21). The fastest colony formation and proliferation was observed in the media containing 10% platelet rich plasma. These results confirm that PRP enhances MSC proliferation as described in some earlier studies (50, 51). We have not used any higher concentration of FCS and human blood components than 10%. In our earlier study (unpublished data) we had already proven higher concentrations are cytotoxic and led to senescence of DPSC lineage. DPSC cultivated in media containing 10% PRP proliferate faster, on the other hand DPSC cultivated in media containing 10% HP did not proliferate at all. Media containing 2% HP or 2% PRP enriched by ITS, EGF and PDGF showed similar proliferation activity, which was slower than in case in media of 10% PRP. We conclude that those three supplements support the cells enough to keep proliferation, but still there are more important growth factors which enhanced the proliferation rate. The DPSC cultivated in 10% PRP grew even faster than the ones cultivated in 2% FCS. In our previous studies we had proven that the DPSC cultivated in a media containing 2% FCS proliferate faster than those cultivated in media containing 10% FCS (43), thus we conclude, that DPSC cultivated in 10% PRP media proliferate faster than DPSC cultivated in 10% FCS and therefore this medium seems to be optimal. We didn't observe any significant visible differences between the DPSC cultured in media with 2% FCS, 2% HP and 2% PRP. We suggest that the cause of this result is the comparable amount of growth factors in the

Tab. 2: The positivity of the DPSC's CD cultivated in tested media. We can see decreasing positivity for CD44, CD73, CD90, CD105, CD146 in DPSC cultivated in media supplemented with PRP. . We can see decreasing positivity for CD44, CD73, CD90, CD105, CD146 in DPSC cultivated in media supplemented with PRP. The decreasing positivity is connected with increasing concentration of PRP in cultivation media.

	CD29	CD44	CD73	CD90	CD105	CD146
Medium 1	86.1%	41.7%	67.81%	82.15%	26.92%	63.94%
Medium 2	81.09%	64.78%	73.62%	85.49%	38.28%	68.66%
Medium 4	89.49%	33.26%	34.94%	49.24%	10.2%	40.45%
Medium 5	98.56%	10.66%	12.77%	12.05%	5.12%	41.72%

media; either added or released from blood platelets. Our results correlate with the results of studies made on DPSC cultivated in the presence of PRP (19, 20, 21).

The differences among the expression of individual epitopes on the cell surface were very significant between the group of cells cultured in the media containing 2% FCS and 2% HP and the cells cultured in the media containing 2% and 10% PRP. Compared to the cells cultured in the 2% FCS and 2% HP media the expression of the markers CD44, CD73, CD90 and the CD105 was lower on the DPSC cultivated in 2% PRP media and the lowest positivity showed DPSC cultivated in 10% PRP. Conversely the CD29 and CD146 markers expression were analogously high in all the media, with exclusion of the CD146 marker expression in PRP cells, where it was moderate. These findings do not correlate with the studies made by Govindasamy, who did not observe any significant changes. The reason for this disparity can be the fact that we cultivated the DPSC in the PRP medium for 5 passages before phenotypic analysis, in the study made by Govindasamy the analysis was done in passage No. 2 (21).

Conclusions

PRP in concentration of 10% highly increased the proliferation activity, but on the other hand PRP affected the phenotype of the cultivated DPSC. Further study of this effect is necessary, mostly wider panel of tested CD has to be compared and the differentiation capacity has to be proved. HP which was not substitute by the growth factors did not support DPSC enough and the lineage was slowly lost. The experiment has successfully proven that DPSC can be cultivated in cultivation media where the FCS was replaced by human blood components and thus this xenogeneic component can be substitute by allogeneic or better autologous blood components.

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Conflict of interest statement

Author's conflict of interest disclosure: None declared.

References

- Gronthos S, et al. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences* 2000; 97(25): 13625–30.
- Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002; 81: 531–35.
- Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, et al. A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). *J Bone Miner Res* 2005; 20: 1394–402.
- Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. *Tissue Eng* 2006; 12: 2813–23.
- Honn KV, Singley JA, Chavin W. Fetal bovine serum: A multivariate standard. *Proc Soc Exp Biol Med* 1975; 149: 344–47.
- Zheng X, Baker H, Hancock WS, et al. Proteomic analysis for the assessment of different lots of fetal bovine serum as a raw material for cell culture. Part IV. Application of proteomics to the manufacture of biological drugs. *Biotechnol Prog* 2006; 22: 1294–300.
- Martin MJ, Muotri A, Gage F, et al. Human embryonic stem cells express an immunogenic nonhuman sialic acid. *Nat Med* 2005; 11: 228–32.
- Spees JL, Gregory CA, Singh H, et al. Internalized antigen must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. *Mol Ther* 2004; 9: 747–56.
- Halme DG, Kessler DA. FDA regulation of stem-cell-based therapies. *New Engl J Med* 2006; 355: 1730–35.
- Medicinal and other products and human and animal transmissible spongiform encephalopathies: Memorandum from a WHO meeting. *Bull World Health Organ* 1997; 75: 505–13.
- Heiskanen A, Satomaa T, Tiitinen S, et al. N-glycolylneuraminic acid xenoantigen contamination of human embryonic and mesenchymal stem cells is substantially reversible. *Stem Cells* 2007; 25: 197–202.
- Chachques JC, Herreros J, Trainini J, et al. Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillator in cellular cardiomyoplasty. *Int J Cardiol* 2004; 95(Suppl 1): S29–S33.
- Tuschong L, Soenen SL, Blaese RM, et al. Immune response to fetal calf serum by two adenosine deaminase-deficient patients after T cell gene therapy. *Hum Gene Ther* 2002; 13: 1605–10.
- Bruinink A, Tobler U, Halg M, et al. Effects of serum and serum heat-inactivation on human bone derived osteoblast progenitor cells. *J Mater Sci Mater Med* 2004; 15: 497–501.
- Dictus C, Tronnier V, Unterberg A, et al. Comparative analysis of in vitro conditions for rat adult neural progenitor cells. *J Neurosci Methods* 2007; 161: 250–58.
- Dimarakis I, Levicar N. Cell culture medium composition and translational adult bone marrow-derived stem cell research. *Stem Cells* 2006; 24: 1407–08.
- Sekiya I, Larson BL, Smith JR, et al. Expansion of human adult stem cells from bone marrow stroma: Conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells* 2002; 20: 530–41.
- Stojkovic P, Lako M, Przyborski S, et al. Human-serum matrix supports undifferentiated growth of human embryonic stem cells. *Stem Cells* 2005; 23: 895–902.
- Lee U, Jeon SH, Park J, Choung P. Effect of platelet-rich plasma on dental stem cells derived from human impacted third molars. *Regen Med* 2011 Jan; 6(1): 67–79.
- Chen B, Sun H, Wang H, Kong H, Chen F, Yu Q. The effects of human platelet lysate on dental pulp stem cells derived from impacted human third molars. *Biomaterials* 2012 Jul; 33(20): 5023–35.
- Govindasamy V, Ronald VS, Abdullah ANB, Nathan KRG, Aziz ZACHA, Abdullah M, et al. Human platelet lysate permits scale-up of dental pulp stromal cells for clinical applications. *Cytotherapy* 2011 Nov; 13(10): 1221–33.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006; 101: e45–50.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006; 101: e51–5.
- Mannello F. Serum or Plasma Samples? The “Cinderella” Role of Blood Collection Procedures Preanalytical Methodological Issues Influence the Release and Activity of Circulating Matrix Metalloproteinases and Their Tissue Inhibitors, Hampering Diagnostic Trueness and Leading to Misinterpretation. *Arterioscler Thromb Vasc Biol* 2008; 28: 611–14.
- Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent* 2001; 10(4): 225–8.
- Shahdadfar A, Tronsdal K, Haug T, et al. In vitro expansion of human mesenchymal stem cells: Choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. *Stem Cells* 2005; 23: 1357–66.
- Stute N, Holtz K, Bubenheim M, et al. Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. *Exp Hematol* 2004; 32: 1212–25.
- Lin HT, Targn YW, Chen YC, et al. Using human plasma supplemented medium to cultivate human bone marrow-derived mesenchymal stem cell and evaluation of its multiple-lineage potential. *Transplant Proc* 2005; 37: 4504–05.
- Kobayashi T, Watanabe H, Yanagawa T, et al. Motility and growth of human bone-marrow mesenchymal stem cells during ex-vivo expansion in autologous serum. *J Bone Joint Surg Br* 2005; 87: 1426–33.
- Mizuno N, Shiba H, Ozeki Y, et al. Human autologous serum obtained using a completely closed bag system as a substitute for fetal calf serum in human mesenchymal stem cell cultures. *Cell Biol Int* 2006; 30: 521–24.

31. Muller I, Kordowich S, Holzwarth C, et al. Animal serum-free culture conditions for isolation and expansion of multipotent mesenchymal stromal cells from human BM. *Cytotherapy* 2006; 8: 437–44.
32. Hilfiker A, Kasper C, Hass R, Haverich A. Mesenchymal stem cells and progenitor cells in connective tissue engineering and regenerative medicine: is there a future for transplantation?. *Langenbecks Arch Surg* 2011; 396: 489–97.
33. Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* 2010; 466: 829–34.
34. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal* 2011; 9: 1–14.
35. Mannello F, Gaetana AT. 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* 2007; 25(7): 1603–09.
36. Spees JL, Gregory CA, Singh H, et al. Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. *Mol Ther* 2004; 9: 747–56.
37. Chachques JC, Herreros J, Trainini J, et al. Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillators in cellular cardiomyoplasty. *Int J Cardiol* 2004; 95(Suppl 1): S29–S33.
38. Hemeda H, Giebel B, Wagner W. Evaluation of human platelet lysate versus fetal bovine serum for culture of mesenchymal stromal cells. *Cytotherapy* 2014; 16(2): 170–80.
39. Gstraunthaler G, et al. Human Platelet Lysates Successfully Replace Fetal Bovine Serum in Adipose-Derived Adult Stem Cell Culture. *Journal of Advanced Biotechnology and Bioengineering* 2014; 2(1): 1–11.
40. Mojica-Henshaw MP, et al. Serum-converted platelet lysate can substitute for fetal bovine serum in human mesenchymal stromal cell cultures. *Cytotherapy* 2013; 15(12): 1458–68.
41. Trojahn Kölle S-F, et al. 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* 2013; 15(9): 1086–97.
42. Stute N, et al. Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. *Experimental Hematology* 2004; 32(12): 1212–25.
43. Suchanek J, et al. Dental pulp stem cells and their characterization. *Biomedical Papers of the Medical Faculty of Palacky University in Olomouc* 2009; 153(1): 31–35.

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