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# Hybrid Machine Learning Approaches in Viability Assessment of Dental Pulp Stem Cells Treated with Platelet-Rich Concentrates on Different Periods

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**Abstract**
The unique characteristics of dental pulp stem cells (DPSCs), like multi-lineage differentiation, have attracted considerable interest among clinicians and researchers for the treatment of various diseases. Platelet-derived concentrates (PRSs) are utilized for wound healing, due to the plethora of growth factors that are released from platelets. In this study, DPSCs were cultured with one of the three culture supplements, including fetal bovine serum (FBS), human platelet-rich plasma (PRP), and human platelet lysate (HPL). The viability effects of these platelet-derived culture supplements on DPSCs were evaluated using hybrid approaches of fuzzy-genetic methods. The results showed that DPSCs cultured in HPL have higher viability than FBS and PRP. It is suggested that fuzzy-genetic algorithm (GA) is an accurate approach to estimate the effect of platelet concentrates on the proliferation of stem cells derived from the human tooth.

**Keywords:** Dental pulp stem cells; Human platelet-rich concentrates; Fuzzy-Genetic Algorithm GA; Proliferation

## Introduction

Tissue regeneration is based on the concept of restoring the physical integrity of cells, tissues, and organs by means of the organisms' own repair mechanisms. A successful regenerative therapy involves a sufficient population of stem cells and the plethora of enzymes, signal proteins, ligands, and a functional vasculature [1]. Clinical studies in the past have reported mesenchymal stem cells (MSCs) to be safe for therapeutic use [2]. Dental pulp stem cells (DPSCs) are one such type of easily available MSCs which have a non-invasive mode of isolation and hold great potential to be used in regenerative protocols. DPSCs have higher angiogenic, neurogenic, and regenerative potential, presenting a versatile stem cell source for cellular therapies [3]. In dentistry, DPSCs have

demonstrated the ability to differentiate into odontoblasts under stimulation of bioactive materials that makes them a potential source of stem cells for dentine and pulp regeneration [4]. They can be easily collected from extracted healthy or inflamed permanent teeth and harvested in a minimally invasive and safe manner. In order for stem cell therapy to be effective, cells should be in sufficient numbers, usually in the order of millions. In the majority of clinical trials, commercially available fetal bovine serum (FBS) is used for stem cells isolation and expansion. It provides a rich composite of growth factors to yield sufficient cell proliferation and expansion during cell culture experiments [5]. However, it does pose a risk of immunogenic reactions in response to xenogeneic proteins [6]. Hence, it is of paramount importance to explore the alternatives that are cost-effective, autologous, prion free in nature and can effectively improve the proliferation and differentiation of the stem cells in-vitro.

*Literature Review and Motivation of the Present Study*

A recent strategy in many regenerative fields is the use of platelet-rich concentrates (PRCs). In general, PRCs are concentrated sources of autologous platelets or their extracts in a small amount of plasma [7]. Several studies have shown that stem cells expanded in two-dimensional cultures using PRCs uphold their multi-potency and therapeutic properties [8] and indicated no signs of immunogenic reactions when used in clinical trials [9]. Translation of the combination of stem cell and PRCs in clinical set up is being powered by their easy availability, cost-effectiveness, an extensive range of applications, autologous nature, simple collection, easy chair side preparation, and clinical application without the risks associated with allogeneic products [10]. Statistical and machine learning methods are playing an important role in the development of a robust decision support system in medical science at present [11]. Some of the recent research includes an application of deep learning-based method in the computer-aided disease diagnosis [12], hierarchical clustering analysis, principal component analysis, and linear modeling in clinical and cognitive data mining [13]. The applications of machine learning methods in dentistry have been discussed in some recent research studies, including semi-supervised fuzzy clustering method in dental image separation [14], the artificial neural network method in the classification of dental cusps (the maximum accuracy of 93.5%) [15], and C4.5 decision trees to predict the microbiological profile dental implants (the maximum accuracy of 97.5%) [16], etc. Genetic algorithm (GA) has been also implemented in different applications of dentistry, like in the optimization of dental milling process [17], analysis of dental images [18], dental implant designing [19], decreasing dental filling metallic artifacts [20], in color matching [21], etc. Though, there are limited studies based on the application of hybrid machine learning methods in medical and specifically in dentistry data mining. The hybrid machine learning methods can result in better prediction accuracy of the decision support system. Fuzzy-GA is one of the hybrid machine learning methods based on the combination of a fuzzy method and the genetic algorithm. It exhibits worthy learning and prediction capabilities that make it a competent and effective tool to manage the encountered uncertainties in any system. Application of fuzzy-GA method does not involve the learning of the physical process as a precondition. With this motivation, fuzzy-GA approaches based on the Mogul and the Thrift approaches [22-24] have been implemented to estimate the effect of human platelet concentrates on human tooth stem cells in the present study.

## Material and Method

*Study Design, Population and Isolation From Human Dental Pulp*

DPSCs were isolated from the dental pulp of the extracted human permanent teeth. Figure 1 depicts the schematic representation of the experimental process. The study was commenced with an isolation of DPSCs from the pulp tissue (n=5) cultured up to passage 3. DPSCs were then treated with two types of PRCs, HPL (human platelet lysate) and PRP (platelet-rich plasma) and were investigated for their effect on morphology, cell viability, and tube formation capacity. Cells cultured in complete media were considered as control.

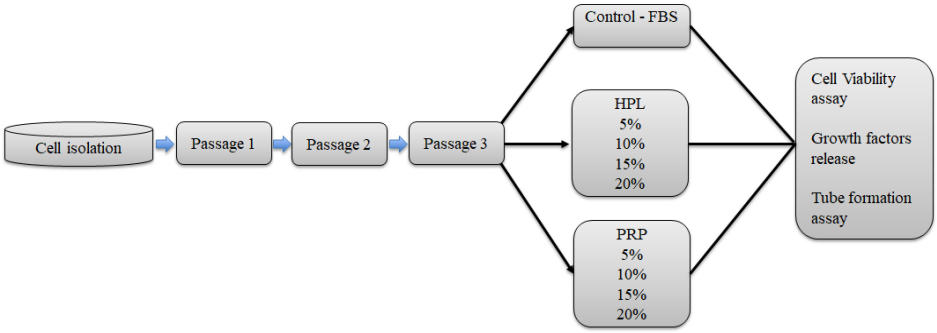


**Figure 1.** A schematic representation of experiment process

Freshly extracted sound premolars were received from the Faculty of Dentistry, University of Malaya following informed written consent obtained from the donors aged 18-25 years undergoing orthodontic treatment. The extirpated pulp tissue (n=5) was processed as per our previously established protocol [25]. The cell suspension cultured in T25 culture flask and incubated in a humidified incubator maintained at 37°C supplemented with 5% $CO_2$. Incubation was carried out till 80% confluence was observed under a phase-contrast microscope.

*Preparation of Platelet Rich Concentrates (PRCs) and Media With Concentrations of HPL and PRP*

PRP samples (n=8) were obtained from the blood bank of the University of Malaya Medical Centre, Kuala Lumpur, Malaysia after ethics approval from the Medical Ethics Committee. PRP was was processed as per our previously established protocol [26] from eight healthy donors kept in triple bags and was stored at room temperature (20°C). 100 ml of PRP from each donor bag was collected in blood collection tubes for research purpose. For the preparation of HPL, PRP from all eight donors was pooled (800 ml) and was processed. The final products were then stored at -80°C until further use. DPSCs (n=5) culture and expansion in various concentrations of HPL and PRP were done as per our previously established protocol [26].

*Evaluation of the Viability of DPSCs in Various Concentrations of HPL and PRP*

DPSCs (n=5) at the 3rd passage, were seeded into 96-well plates in triplicates at a density of $5\times10^3$ cells per well in 200µl of complete culture media. Cells were incubated with various concentrations of PRCs media and complete culture media for 2, 4, 6, 8, and 10 days. After that, 100µl of DMEM-KO (Gibco) along with 10% Alamar Blue reagent (Thermo Fisher Scientific, USA) v/v was added to each well and the plates were further incubated for 3 hours at 37°C and 5% $CO_2$ in a humidified incubator. Absorbance was measured at 450nm with a reference wavelength (590nm) by Tecan (Infinite 200 PRO, Life Sciences, Switzerland) microplate reader. The viability of DPSCs after treatment with HPL and PRP was determined using Alamar Blue assay (Thermo Fisher Scientific). The cell viability was calculated in percentage of the reduction in the recorded absorbance. DPSCs cultured in media supplemented with FBS is considered as control and reference (normalized to 100%).

*Hybrid Fuzzy-GA Methods*

Two types of hybrid fuzzy-GA methods have been implemented, including (i) Mogul method based fuzzy-GA [22], and (ii) Thrift approach based fuzzy-GA [23]. The ideal boundary values of a fuzzy method in each type were decided by using the GA. The boundary values were restructured in every cycle of the GA. Initially, a random population for GA was selected inside the bounding intervals of fuzzy. GA monitors the limits of input variables and the progression of variables. The preliminary population of GA was employed to prepare the knowledge base (KB), which was further amended in the succeeding cycles of GA. The Mogul method based fuzzy-GA develops the membership function parameters and fuzzy rules using the GA [22]. The method contains three steps, including (i) rule base (RB) preparation, (ii) genetic interpretation, and (iii) genetic modification.

Comprehensive information from the training data set was extracted and used in the RB generation. The RB was summarized in the genetic simplification step by discarding the impractical rules. The precision of the KB was enhanced in the genetic tuning step. The initial fuzzy rules containing the basic evidence of the membership function were used as the primary population of the GA. The genetic operations were implemented amongst the parent chromosomes to create the subsequent generation offspring and continued till the end criterion is attained. This procedure recovers the superiority of chromosomes and lastly, the best chromosomes were chosen as the fuzzy rules to design the ideal fuzzy-GA method [22]. The Thrift method based fuzzy-GA employs Mamdani models of fuzzy reasoning and GA in which the decision was coded into the chromosomes. It maps the output variable into an integer set; the latter comprises a zero and a non-zero element to describe the gene set [23]. The GA uses an integer coding pattern to generate the chromosomes. The existence of the Mamdani model for fuzzy reasoning in Thrift method based fuzzy-GA makes it different than the Mogul method based fuzzy-GA, however, the selection procedure of initial chromosomes was similar in both types. The crossover and mutation operators produce novel sets of chromosomes in successive cycles of GA. Root mean square error (RMSE) was assumed to calculate the fitness values of each of the chromosomes in each cycle. The best chromosomes were employed as the ideal fuzzy rules in the Thrift method based fuzzy-GA, which forecast the bordering values of the dependent variable. The hybrid fuzzy-GA approaches have been implemented in R language [24]. Predictive performances of fuzzy-GA methods have been measured in terms of three evaluation metrics, including RMSE, the coefficient of determination ($R^2$), and the Pearson correlation coefficient (r).

## Results

*Viability of DPSCs after Treatment With Various Concentrations of HPL and PRP*

Isolated MSCs from dental pulp tissue as represented in Figure 2 were expanded till the third passage and further treated with various concentrations of HPL and PRP.

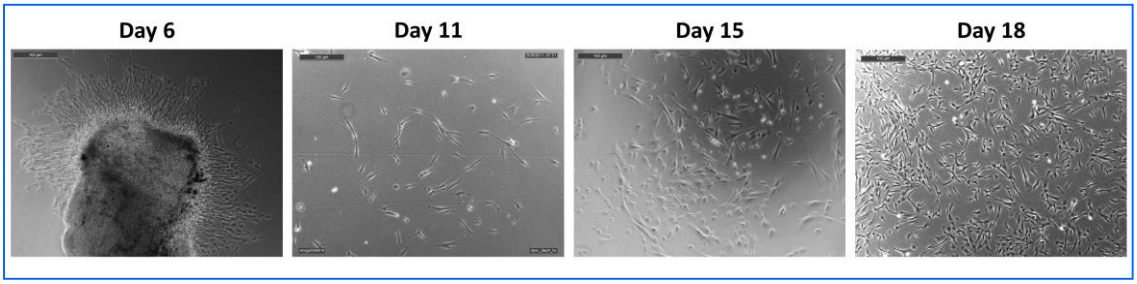


**Figure 2.** Isolated mesenchymal stem cells from dental pulp tissue

Morphology of DPSCs treated with various concentrations of HPL and PRP are depicted in the micrographs presented in Figure 3. Morphology of treated DPSCs started to transform from fibroblastic (spindle shaped) to flattened morphology.

Cell viability was measured for ten days, and observations were recorded at an interval of 2 days as represented in Figure 4. Significantly ($p<0.05$) higher viability was observed in DPSCs treated with 10, 15, and 20% of HPL compared to that of PRP. However, no significant difference in viability was observed among the DPSCs treated with 5% HPL, 5% PRP, and 10% PRP. The viability of DPSCs in both the groups was significantly lower at day 6 ($p<0.05$) compared to the viability at day 2, 4, 8, and 10 at all concentrations. In addition, an interesting pattern was observed in all tested concentrations whereby the viability decreased from day two until day six of culture before an increase by day 10. A significantly higher ($p<0.05$) viability was observed with HPL 10%, 15% and 20% at day 2 (97%) compared to day 6 (64%). Moreover, the viability of DPSCs treated with HPL was significantly increased ($p<0.05$) at day 10 compared to that on day 6. Except at 5%, HPL (~70%) viability of DPSCs in other concentration was about 80%. In PRP irrespective of the concentration and time, the viability of DPSCs was equal to 80% or below. Viability varied the most amongst

various concentrations of PRP. In PRP treated cells, it was especially noted that increasing its concentration more than 5% did not alter the final viability of cells. Exposure of DPSCs to various concentrations of HPL and PRP (10% and 20%) at day 2, 4, 6, 8, and day 10 showed significantly ($p<0.05$) high viability at day 2, and day 4 for 10%, 15%, and 20% HPL media groups.

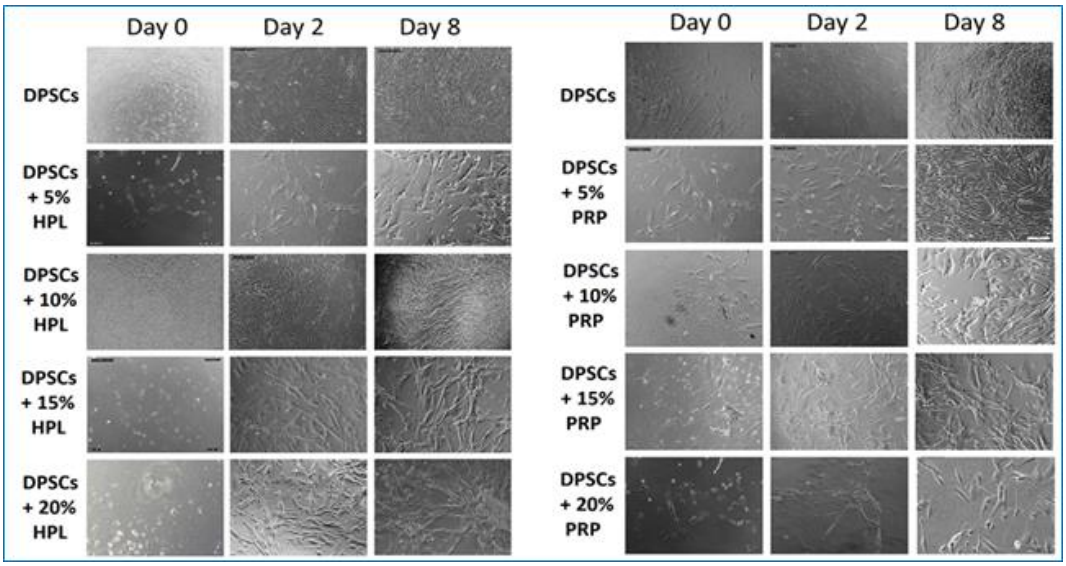


**Figure 3.** Morphology of DPSCs after treatment with HPL and PRP

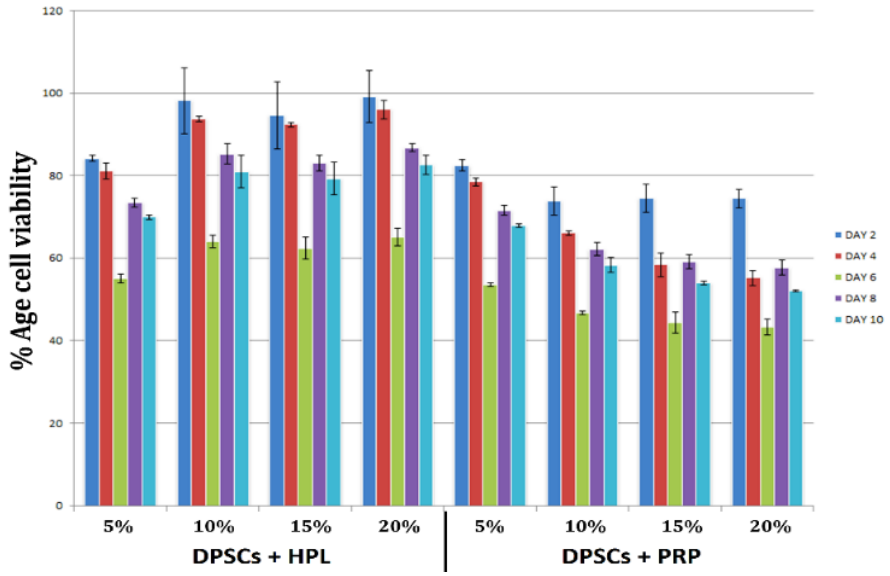


**Figure 4.** Percentage of cell viability upon exposure to various concentrations of HPL and PRP at five time intervals

*Prediction Results of Hybrid Fuzzy-GA Methods*

Figure 5 (a) and Figure 5 (b) exhibit the viability prediction results of HPL and PRP using two fuzzy-GA methods, respectively. The higher value of the coefficient of determination in Table 1 confirms the better performance of the methods. There is a smaller number of underestimated and overestimated samples. Consequently, the predicted values have a high level of precision. Mogul method based fuzzy-GA outperforms Thrift method based fuzzy-GA. With the objective to demonstrate the assets of the suggested model on a more confident and noticeable basis, statistical

measures, including the RMSE, r, and $R^2$ were computed and compared for the two fuzzy-GA algorithms. Table 1 summarizes the prediction accuracy results.

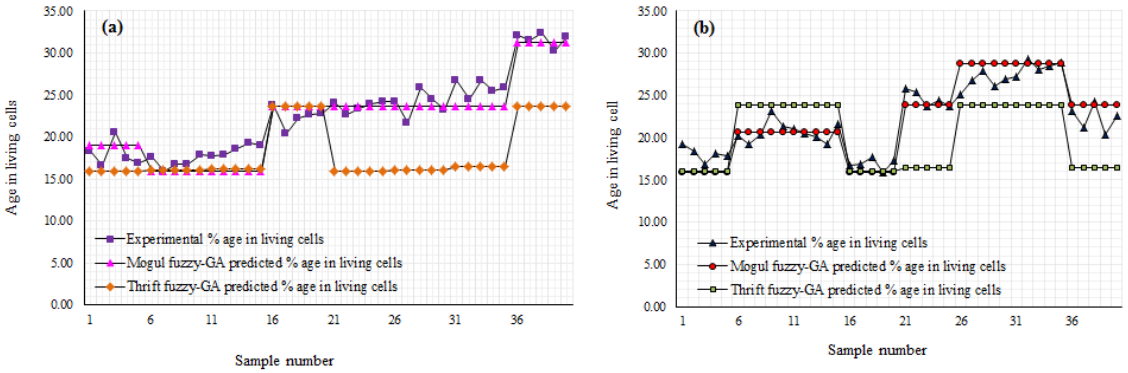


**Figure 5.** Fuzzy-GA prediction results of % age in living cells cultured in (a) HPL and (b) PRP

**Table 1.** Culture media groups used in the experiment

| Accuracy measures | Mogul method based fuzzy genetic algorithm (GA) | | Thrift method based fuzzy genetic algorithm GA | |
|---|---|---|---|---|
| | PRP | HPL | PRP | HPL |
| r | 0.94 | 0.94 | 0.48 | 0.56 |
| $R^2$ | 0.89 | 0.89 | 0.23 | 0.32 |
| RMSE | 1.64 | 1.68 | 4.52 | 6.04 |

Note-RMSE: root mean square error, PRP: platelet-rich plasma, HPL: human platelet lysate.

## Discussion

As depicted in Figure 1, PRCs namely HPL and PRP are extracted from human blood plasma after different processing techniques and appropriate pathogen inactivation. PRCs demonstrate potential cell growth-promoting and differentiation activity with reduced chances of infection transmission compared to animal-based serums. PRCs have also been demonstrated as an effective source of angiogenesis promoting growth factors (GFs), which is a vital requisite for the healing of inflamed tissue. Despite the reported application of PRP, there are some shortcomings, according to its clinical use, growth factors diversity and concentration, temporal degradation, and inadequate mechanical strength [27]. PRCs are known to contain a plethora of GFs and could have an augmented effect on the angiogenic potential of DPSCs. However, the effect of PRCs on DPSCs has not been explored widely. Hence, this study was designed to address the suitable concentration of HPL and PRP to maintain the viability of DPSCs, comparable to FBS, *in vitro* (Figures 2-4). Since pooling of PRP from various donors reduces the individual variation, in the present study, HPL was prepared by freezing and thawing of pooled PRP from eight healthy donors. A previous study [25], reported that both 10% and 20% of the platelet lysate (HPL) support cell viability and migration in a co-culture of endothelial cells, monocytes, fibroblasts, and keratinocytes. From the findings of previous study [25] a range of HPL and PRP concentrations was selected to optimize the most suitable concentration to support the DPSCs viability (Figures 2-4). It was observed that 20% PRP did not show a higher viability compared to other concentrations of HPL and PRP at the 48$^{th}$ hour (Figure 4). This might be due to the fact that PRP requires biological activation to initiate efficient release of growth factors from the platelets. There was an initial release of growth factors due to the degranulation of platelets, which might have occurred due to the interaction with culture media components and repeated physical manipulation during the experiments. Hence in the case of PRP, growth factors might not

be in active state in media as compared to HPL where growth factors are readily present in active state in media. We also speculated that PRP could not reach its full potential in terms of releasing active growth factors. Another study on periodontal ligament (PDL) fibroblast demonstrated significantly higher cell viability when PDL fibroblasts were treated with 5% PRP compared to 10% and 50% PRP [28]. It was confirmed that remarkably reduced viability was evident at 5% PRP. The optimized concentrations were then investigated for their growth promoting effect on DPSCs. After culturing DPSCs in various concentrations of HPL and PRP, the morphology of DPSCs was observed to be elongated and spindle-shaped (Figure 3). There were no significant visible differences in morphology between the DPSCs cultured in media with HPL and PRP when compared to those cultured in media supplemented with FBS. These findings were partially in line with a study done on the cryoprotective effect of PRP [9]. It was also observed that at 20% HPL concentration, DPSCs started expanding and becoming flat; this closely resembles the morphology of endothelial cells (Figure 3). However, no studies in the literature have reported on the effect of higher concentrations of HPL on DPSCs morphology. The cell viability results of the present study showed that HPL at 10%, 15%, and 20% supported the higher cell viability especially at the 48$^{th}$ hour and was found to be comparable to FBS (Figure 4). This observation was in agreement with a study done on adipose-derived stem cells [29]. Higher viability was noticed in HPL treated DPSCs when compared to PRP; however, PRP at 5% concentration showed a good percentage of viable cells on day 2 and day 4. This viability promoting effect at was observed at lower concentrations of PRP and viability reducing effect was observed at higher PRP concentrations. These findings were in line with a similar study performed on BMSCs treated with various concentrations of platelet concentrates [8]. The viability results of our study were also supported by another study suggesting a higher cell viability effect of 10% and 20% HPL on a co-culture of endothelial cells, monocytes, fibroblasts and keratinocytes [10]. From the findings of an earlier study [26], it can be suggested that the effect of HPL and PRP on cell viability was dose-dependent, and 10%, 15% and 20% HPL, and 5% PRP hold the potential of replacing FBS as a media supplement in DPSCs culture. On comparing the effect of treatment time of HPL and PRP on cell viability of DPSCs, a general decrease in viability of DPSCs was observed from day 2 to day 6 that later exhibited an incremental pattern till day 10 (Figure 4). The Mogul fuzzy-GA predicted values of the % age in the living cell cultured in PRP are closer to their experimental measured values than the cell cultured in PRP. It is obvious from Figure 5 (b) and evaluation measures in Table 1. Moreover, the straight evaluation of analysis results of fuzzy-GA approaches is not possible due to the unavailability of any previous study for the viability assessment of dental pulp stem cells.

The limitation of the present study includes the scalability of stem cells in vitro culture and short life of HPL which is efficient for chair side procedures but has limited use for long term therapies. However, PRP can be used for long term therapies. Due to the autologous nature of HPL and PRP, there are negligible chances of immune rejection.10% HPL is suitable to replace FBS in the expansion of stem cells scheduled for clinical trials. HPL and PRP contain a cocktail of growth and it is a potential source of regenerative media for inflamed dental pulp that has limited blood supply or other regenerative studies involving other parts of the human body such as the knee and ischemic cardiovascular tissue release active growth factors. The fuzzy-GA approach results in a better decision support system for the prediction of age in living cells cultured in HPL and PRP.

## Conclusions

Based on the results of the present study, DPSCs cultured in HPL have higher viability than FBS and PRP. HPL at 10%, 15%, and 20% supported the higher cell viability and was found to be comparable to FBS. However, no significant difference in viability was observed among the DPSCs treated with 5% HPL, 5% PRP and 10% PRP. In this study, the Mogul method surpasses the Thrift method. The hybrid fuzzy-genetic algorithm could be proposed as a modern machine learning approach with high prediction accuracy to estimate the effect of human platelet concentrates on the proliferation of stem cells derived from the human tooth.

## Ethical Issues

Ethical approval has been obtained from the Medical Ethics Committee, Faculty of Dentistry, and University of Malaya [DFDP 1304/002 (P)] for the collection of human extracted permanent teeth samples and their processing. Authors affirm that the content of this manuscript is original. It has been neither published elsewhere nor submitted for publication simultaneously.

## Conflict of Interest

No conflict of interest.

## Acknowledgements

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## References

1. Jung S, Kleinheinz J. Chapter 19. Angiogenesis-the key to regeneration. In: Andrades JA. (Ed) Regenerative Medicine and Tissue Engineering. IntechOpen. 2013, pp. 453-473. doi:10.5772/55542
2. Sotiropoulou PA, Papamichail M. Immune properties of mesenchymal stem cells. Methods in Molecular Biology 2007;407:225-243.
3. Iohara K, Murakami M, Takeuchi N, Osako Y, Ito M, Ishizaka R, et al. A novel combinatorial therapy with pulp stem cells and granulocyte colony stimulating factor for total pulp regeneration. Stem Cells Translational Medicine 2013;2(7):521-533.
4. Prescott RS, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J, et al. In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. Journal of Endodontics 2008;34(4):421-426.
5. Bettiol E, Sartiani L, Chicha L, Krause KH, Cerbai E, Jaconi ME. Fetal bovine serum enables cardiac differentiation of human embryonic stem cells. Differentiation 2007;75(8):669-681.
6. Van der Valk J, Brunner D, De Smet K, Fex Svenningsen A, Honegger P, Knudsen LE, et al. Optimization of chemically defined cell culture media–replacing fetal bovine serum in mammalian in vitro methods. Toxicology in Vitro 2010;24(4):1053-1063.
7. Ehrenfest DM, Andia I, Zumstein MA, Zhang C, Pinto NR, Bielecki T. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. Muscles, Ligaments and Tendons Journal 2014;4(1):3-9.
8. Crespo-Diaz R, Behfar A, Butler GW, Padley DJ, Sarr MG, Bartunek J, et al. Platelet lysate consisting of a natural repair proteome supports human mesenchymal stem cell proliferation and chromosomal stability. Cell Transplantation 2011;20(6):797-812.
9. Ramos-Torrecillas J, De Luna-Bertos E, García-Martínez O, Ruiz C. Clinical utility of growth factors and platelet-rich plasma in tissue regeneration: a review. Wounds 2014;26(7):207-213.
10. Schär MO, Diaz-Romero J, Kohl S, Zumstein MA, Nesic D. Platelet-rich concentrates differentially release growth factors and induce cell migration in vitro. Clinical Orthopaedics and Related Research 2015;473(5):1635-1643.
11. Becker NG. Analysis of infectious disease data. Routledge; 2017. Available from: URL: https://doi.org/10.1201/9781315137407.

12. Shen D, Wu G, Suk HI. Deep learning in medical image analysis. Annual Review of Biomedical Engineering 2017;19:221-48.
13. Ramanan S, de Souza LC, Moreau N, Marie S, Antônio LT, Zoe A, et al. Determinants of theory of mind performance in Alzheimer's disease: a data-mining study. Cortex 2017;88:8-18.
14. Tuan TM. A cooperative semi-supervised fuzzy clustering framework for dental X-ray image segmentation. Expert Systems with Applications 2016;46:380-393.
15. Raith S, Vogel EP, Anees N, Christine K, Jan-Frederik G, Daniel E, et al. Artificial Neural Networks as a powerful numerical tool to classify specific features of a tooth based on 3D scan data. Computers in Biology and Medicine 2017;80:65-76.
16. Canullo L, Radovanović S, Delibasic B, Juan AB, David P, Mia R. The predictive value of microbiological findings on teeth, internal and external implant portions in clinical decision making. Clinical Oral Implants Research 2017;28(5):512-519.
17. Vera V, Corchado E, Redondo R, Sedano J, Garcia AE. Applying soft computing techniques to optimise a dental milling process. Neurocomputing 2013;109:94-104.
18. Majdi-Nasab N, Analoui M, Delp EJ. Decomposing parameters of mixture Gaussian model using genetic and maximum likelihood algorithms on dental images. Pattern Recognition Letters 2006;27(13):1522-1536.
19. Roy S, Dey S, Khutia N, Chowdhury AR, Datta S. Design of patient specific dental implant using FE analysis and computational intelligence techniques. Applied Soft Computing 2018;65:272-279.
20. Abdoli M, Ay MR, Ahmadian A, Dierckx RA, Zaidi H. Reduction of dental filling metallic artifacts in CT-based attenuation correction of PET data using weighted virtual sinograms optimized by a genetic algorithm. Medical Physics 2010;37(12):6166-6177.
21. Li H, Lai L, Chen L, Lu C, Cai Q. The prediction in computer color matching of dentistry based on GA. Computational and Mathematical Methods in Medicine 2015;2015:1-7.
22. Herrera F, Lozano M, Verdegay JL. A learning process for fuzzy control rules using genetic algorithms. Fuzzy Sets and Systems 1998;100(1-3):143-158.
23. Thrift PR. Fuzzy logic synthesis with genetic algorithms. In ICGA 1991, pp. 509-513.
24. Riza LS, Bergmeir CN, Herrera F, Benítez Sánchez JM. frbs: Fuzzy rule-based systems for classification and regression in R. American Statistical Association. 2015;65(6):30 pages. doi: 10.18637/jss.v065.i06
25. Bindal P, Ramasamy TS, Kasim NHA, Gnanasegaran N, Chai WL. Immune responses of human dental pulp stem cells in lipopolysaccharide-induced microenvironment. Cell Biology International 2018;42(7):832-840.
26. Bindal P, Gnanasegaran N, Bindal U, Haque N, Ramasamy TS, Chai WL, et al. Angiogenic effect of platelet-rich concentrates on dental pulp stem cells in inflamed microenvironment. Clinical Oral Investigations. 2019. doi:10.1007/s00784-019-02811-5
27. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. Platelets 2017;28(8):774-778.
28. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry–part I: stem cell sources. Journal of Prosthodontic Research 2012;56(3):151-165.
29. Gstraunthaler G, Lindl T. Standardisierung in der Zellkultur (Good cell culture practice). In: Zell-und Gewebekultur. Berlin: Springer Spektrum 2013: 147-148.