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41 Growth Factor Array Release Kinetics for PRF and AFG Over Time Show Peak Levels at 1.5 Hours

by
Jin Wan Kim

DISSERTATION
Submitted in partial satisfaction of the requirements for degree of
MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

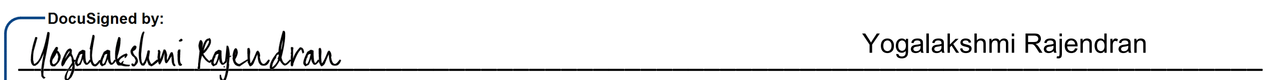
in the

GRADUATE DIVISION
of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

Yvonne Kapila

Chair



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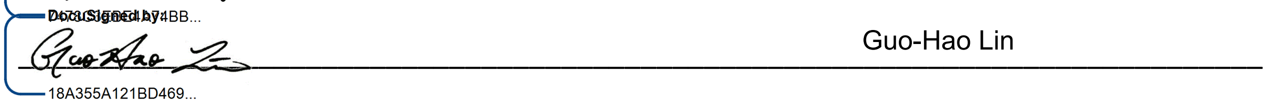

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Acknowledgements

I want to thank Dr. Yvonne Kapila (University of California, San Francisco) and Dr. Yogalakshimi Rajendran (University of California, San Francisco) for providing guidance for the study. I want to also thank Dr. Pachiyappan Kamarajan (University of California, San Francisco) and Dr. Allan Radaic (University of California, San Francisco) for their support in the laboratory technical evaluation and analysis of the results. I want to thank Ahmed Alsaleh and Keun Hwan Kim in the aid in data collection and analysis of the data. Finally, I want to thank The Dr. Benjamin Dienstein Fund for providing the funding for the project.

41 Growth Factor Array Release Kinetics for PRF and AFG Over Time Show Peak Levels at 1.5 Hours

Jin Wan Kim

Abstract

Since Choukroun introduced the use of platelet-rich fibrin (PRF) for oral surgical procedures in 2000, PRF has been researched as autologous biologic material to aid in accelerated healing, regulation of inflammation and surgical site integrity.[2] Autologous fibrin glue (AFG) was introduced by Sohn in 2010 to fabricate a growth-factor-enriched bone graft matrix, also called sticky glue, for bone augmentation.[14] Currently, PRF and AFG are widely used in maxillofacial surgeries to improve healing and predictability of procedures.[1; 3; 4; 8-11; 26] Various studies evaluated the release kinetics of specific growth factors (PDGF-BB, TGFβ-1, VEGF and IGF-1) from PRF.[11-13; 20; 21] However, an evaluation of an extensive array of available growth factors in PRF and AFG has not been conducted. This study evaluated the presence of 41 human growth factors within PRF and AFG and their change over time over a 3-hour period, replicating the potential handling time associated with usage of PRF in a clinical setting. 3 healthy volunteers were recruited from the UCSF Division of Periodontology. Venous blood from each volunteer was collected in three 10ml glass tube and one 10ml plastic tube and centrifuged at 2700rpm for 13 minutes to obtain 3 tubes of PRF and 1 tube of AFG. Each sample was separated into three equal parts and analyzed using a human growth factor antibody array kit. Samples were evaluated at 0, 1.5 and 3 hours after collection. The array data revealed significant peak level concentrations for several growth factors at 1.5 hour and the presence of high concentrations of IGFBP and MCSF R growth factors. The latter growth factors have not been previously reported in studies of PRF and AFG.

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1. Introduction

In 2000, Choukroun was the first to introduce the use of platelet-rich plasma for oral surgical procedures for enhancement of healing.[2] Since then, many generations of enhancements in the technique resulted in the development of platelet-rich fibrin with improved handling and time-release characteristics.[3; 4; 8-10]

Platelet rich fibrin (PRF) is a fibrin matrix obtained naturally from a patient's own blood. PRF releases platelet cytokines, growth factors, and cells over time as healing occurs and it can accelerate healing, self-regulate inflammation, and act as an adhesive at the surgical site to maintain wound closure.[2-4; 8-12] (**Figure 1**, **left**) Autologous Fibrin Glue (AFG) was introduced in 2010 by Sohn to create growth factor-enriched bone graft matrix (sticky bone) when mixed with particulate bone graft.[14] (**Figure 1**, **right**) AFG is similarly obtained like PRF, but a plastic tube is used instead of a glass tube to prevent coagulation. A combination of PRF and AFG are used widely in oral and maxillofacial surgeries for sinus augmentation, soft tissue augmentation, and bone grafting.[1; 14; 26; 31]

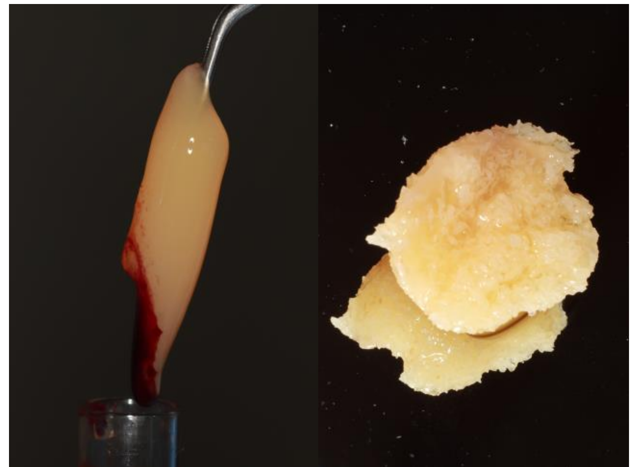

Figure 1. PRF and Sticky Bone (AFG). Left image shows PRF being removed after centrifugation. Right image shows mixture of AFG and freeze-dried allograft to form sticky bone.

Various studies have evaluated PRF release kinetics for specific growth factors, namely PDGF-BB, TGFβ-1, VEGF and IGF-1.[9; 11; 20] However, no studies have evaluated the other extensive array of growth factors that may be released from PRF and AFG, which may be available to enhance healing in the clinical setting. In addition, the potential change in composition of growth factors over time and prior to use is not known. Simultaneous detection of multiple growth factors

allows study of complex and dynamic interactions that may be involved in healing and may help us understand the therapeutic potential of these biomaterials. This study evaluated the release kinetics of a large array of growth factors from PRF and AFG and their changes in composition over a 3-hour period. Another goal of this study was to compare the profile of growth factors released from PRF compared to AFG and whether they differ in composition.

2. Materials & Methods

2.1 Patient population and enrollment

Three medically healthy volunteers (35, 37, and 57 years of age) were recruited from the Division of Periodontology at UCSF. Volunteers with chronic health conditions or smokers were excluded. Volunteers in this study did not receive any incentives to participate, and those that agreed to participate signed consent forms.

2.2 Sample Extraction

From each volunteer, venous blood was collected via venipuncture from the antecubital vein using three 10ml sterile glass tubes and one 10ml sterile plastic tube. The tubes were immediately centrifuged at 2700 rpm for 12 minutes to obtain PRF and AFG using an Intra-Spin centrifuge [Intra-Lock International, Birmingham, AL].[11] After centrifugation, the test tubes were labeled and stored in a bio-hazard container using a double sealing protocol (sealed tubes inside a biohazard container). The container was immediately transferred to the laboratory for the growth factor analysis.

2.3 Sample Preparation

All manipulation of the PRF and AFG samples was conducted in a biosafety level 2 plus laboratory in a laminar flow hood. The PRF from the glass tubes and AFG from the plastic tubes for each

sample were separated from the tubes and placed in individual petri dishes for the PRF and microcentrifuge tubes for the AFG. PRF was obtained from the middle layer within the 3 layers that formed in the glass tubes and AFG was obtained from the top layer within the 2 layers that formed in the plastic tubes. Care was taken not to incorporate any red blood cells from the PRF samples. The PRF was split into 3 equal pieces lengthwise and only the center piece was used for the analysis. At each time point (0 h, 1.5 hour, and 3 hours), the PRF sample was placed into a microcentrifuge tube with 400 µl of radioimmunoprecipitation assay buffer and protease inhibitor cocktail solution (10x dilution) and minced with sterile scissors. The sample was then incubated on ice for 1 hour with agitation every 15 minutes. During this time, the AFG was separated into 3 microcentrifuge tubes and each tube was moved to ice at each time point. No dilution or lysis was performed on the AFG and the serum was used for the total protein analysis. All samples waiting for the next time points were stored at room temperature to replicate clinical use.

2.4 Protein Concentration Assay

To normalize the protein content for all the samples, a protein concentration assay was performed. The total protein concentration in each sample (lysate for PRF and serum for AFG) was determined by spectral analysis [Spectramax M2, Molecular Devices]. In 96 well plates, 25 µl of standards (blank, 25, 125, 250, 500, 750, 1000, 1500, 2000 mg/ml) were placed on the first row and 5 µl of each sample were placed in the subsequent rows and diluted 5-fold with 20 µl of sterile water. Two hundred microliters of a bicinchoninic acid solution were added to each well, then the wells were covered with aluminum foil, and the samples were incubated at 37°C for 30 minutes. After incubation, the bicinchoninic acid assay analysis was performed to obtain the protein concentration for each sample. Each sample concentration was converted to mg/ml to determine the volume needed for 1000 mg of protein in each sample. Samples were diluted with

the blocking buffer provided by growth factor array kit (RayBiotech, Norcross, GA, USA) to obtain 1000 mg/ml concentrations.

2.5 Sample Incubation and Preparation

RayBio® Human Growth Factor Antibody Array C1 (RayBiotech, Norcross, GA, USA), which was stored at -20°C, was normalized to room temperature prior to use. (**Figure 3**) Each array was placed in an incubation tray of 4 x 2 arranged wells and care was taken to arrange the membrane with the same orientation for each sample. Two milliliters of blocking buffer were placed into each well, then the sample-containing plates were incubated for 30 minutes on a tilting laboratory shaker. After aspirating the buffer, 1 ml of each sample was placed into each well, one at a time to avoid drying out the membrane. Once all the samples were placed, wells were incubated overnight at 4°C on a tilting laboratory shaker.

The next day, the samples were brought to room temperature. Then, the sample solutions were aspirated from the wells and the first wash process was conducted. The wash process involved 2 steps: placing 2ml of Buffer I solution in each well and incubating for 5 minutes on the tilting laboratory shaker and this was repeated three times. Then, the same process was conducted with Buffer II and repeated 2 times. After the first wash cycle, a biotinylated antibody cocktail was diluted with 2ml of blocking buffer, then 1ml of this diluted solution was place into each well and incubated for 1.5 hours at room temperature on a tilting laboratory shaker. After incubation, the 2nd wash cycle with the same protocol as the first wash cycle was conducted. Next, HRP-Streptavidin was diluted 1000-fold with blocking buffer. After aspirating the buffer from the 2nd wash cycle, 2ml of the HRP-Streptavidin solution was placed into each well and the samples were incubated for 2 hours at room temperature on a tilting laboratory shaker. A 3rd wash cycle was conducted prior to chemiluminescence detection.

2.6 Chemiluminescence Detection

Figure 2. Antibody Array with Chemiluminescence. Example antibody array with chemiluminescence detection. Both PRF and AFG have many growth factors in common but their concentrations differ shown by different level of intensity of each well.

The membranes in the wells were transported for imaging with the Buffer II solution still present in each well. An Alpha Innotech Fluor Chem Q Multimage III system in the chemiluminescence setting was used for imaging with high resolution. Excess buffer was removed, and membranes were moved to clear plastic sheets one at a time. Detection buffer C and D were mixed in a 1:1 ratio and 500 ml of the solution was dispensed onto each membrane and incubated for 2 minutes at room temperature. Then, a second plastic sheet was placed on top, avoiding bubbles, and membranes were imaged for 1.5-minute exposures. This process was repeated with subsequent arrays and image files were saved in a DNG format. (**Figure 2**)

2.7 Image Analysis and Data Acquisition

Images obtained from the chemiluminescence analyzer were converted using image processing software GIMP [GIMP, GIMP Development Team]. Images were brought into the software, inverted, and the background was normalized to white. Each sample array was selected into a 500x400 pixel rectangle and exported in a PNG format for the image analysis. No other adjustments in exposure, contrast or color were conducted to preserve the raw data. (**Figure 2**)

Exported files from GIMP in PNG format were imported into FIJI with a Protein Array Analyzer software [FIJI, SciJava Projects]. Images were automatically converted to 8bit, and a linear background subtraction at a 25 level was conducted. Arrays of 12x8 were produced with a radius of 12 for each sample position by selecting the top left, top right and lower right corner of the

array. This analysis produced intensity values for each growth factor array and the resulting data was imported into Excel [Microsoft, Redmond, WA] for analysis. Background subtraction using blank wells was conducted and all data were normalized utilizing the positive control and first PRF sample plate selected as a reference array.

2.8 Statistical Analyses

Data was manually imported into GraphPad Prism [GraphPad Software, San Diego, CA] for statistical analysis. Intensity values of each growth factor at each time point for both PRF and AFG was analyzed via 2-way ANOVA and False Discovery Rate was controlled by Benjamini, Krieger and Yekutieli test. Significance was determined by $Q < 0.05$.

3. Results

PRF and AFG was obtained from all three subjects. 41 total growth factors were evaluated for each subject for both PRF and AFG at the three time points of 0-hour, 1.5 hour and 3 hours. Throughout the analysis, 1 AFG growth factor plate from subject #2 at the 3-hour point was determined to have contamination and excluded from the study. After excluding this plate sample, a total of 6 samples were evaluated for each material at each time point due to each growth factor plate having 2 distinct intensity values for evaluation.

Overall, average intensity values indicating relative concentrations for each growth factor demonstrated few appreciable trends. For many of the growth factors analyzed, there was a similar trend showing an increase in growth factor concentration from 0 hour to 1.5 hour followed by a decrease at 3 hours (**Figure 4, Figure 5**). In addition, 7-10 of the growth factors evaluated demonstrate much higher concentration than the rest of the growth factors. For PRF samples, growth factors PDGF-AA, IGFBP-2, PDGF-BB, PDGF-AB, IGFBP-6, MCSF R, EGF

demonstrated the highest concentration followed by PDGF Rb, VEGF R2 and EGF R. For AFG samples, growth factors IGFBP-1, IGFBP-2, IGFBP-6, MCSF R, IGFBP-4, EGF R followed by PDGF Rb, SCF R and VEGF R2 demonstrated the highest concentration.

For the rest of this report, we focus on the top 13 growth factors: EGF, EGF R, IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-6, MCSF R, PDGF-AA, PDGF-AB, PDGF-BB, and PDGF Rb.

Table 1 shows statistical significance for growth factors that demonstrated change in concentration over the 3-hour period. EGF, PDGF-AA, PDGF-AB, and PDGF-BB all demonstrated statistical significance only between 1.5 to 3 hours for PRF. IGFBP-4 demonstrated significant difference in concentration for AFG from 0 hour to 1.5 hour and 1.5 to 3 hour but not for 0 hour to 3 hour. EGF R, IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-6, MCSF R and PDGF Rb did not demonstrate any statistically significant differences at different times.

Table 2 shows growth factors that demonstrate statistical significance between PRF and AFG at specific time points. EGF and IGFBP-4 demonstrated differences between PRF and AFG at all three time points, such that the EFG concentration was higher for PRF and IGFBP-4 was higher for AFG. PDGF-AA demonstrated statistical significance between PRF and AFG at 0 hour and 1.5 hour but not at 3 hours. PDGF-AB and PDGF-BB demonstrated statistical significance between PRF and AFG at 1.5 hour only. EGF R, IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-6, MCSF R and PDGF Rb did not demonstrate any statistically significant differences between PRF and AFG.

4. Discussion

This study evaluated the presence and change of 41 human growth factors in PRF and AFG over time by comparing the level of growth factors in each material over a 3-hour span. By comparing

the levels of different growth factors from PRF and AFG, it may be possible to determine the most suitable applications for PRF and AFG. This study represents the first pilot study to evaluate growth factors not commonly evaluated in studies of PRF and AFG. Also, this study characterized, to a limited extent, the release kinetics of those growth factors over time to replicate a clinical situation.

Most of the current studies evaluating release of growth factors from PRF and AFG focus on release kinetics of days to weeks but have not evaluated changes in growth factors within the first few hours when the biomaterials are utilized.[9; 11; 21; 29] No strict guidelines exist on the timing of the use of the biomaterials after the blood draw is conducted. This study shows that even within the first few hours, many growth factors demonstrate a trend toward increasing levels from 0 to 1.5 hour in a room temperature setting followed by a decrease at the 3-hour mark. Although statistical significance was only observed for EGF, PDGF-AA, PDGF-AB, and PDGF-BB for PRF and IGFBP-4 for AFG, that may be due to a limited number of samples analyzed since a trend is clearly visible in Figure 4 and Figure 5. Interestingly, this trend was more apparent for PRF than AFG.

In addition, few studies have evaluated growth factors available from AFG and whether the composition may be different than the PRF.[29] A study by Serafini et. al evaluated release kinetics of various growth factors (TGF-b1, PDGF-AB, PDGF-BB, BMP-2, FGF-2 and VEGF) from liquid fibrinogen over a 2-week period; liquid fibrinogen is analogous to AFG in this study. Our study did not include evaluation of BMP-2 or FGF-2. However, among the growth factors evaluated, IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-6, MSCF R and EGF R exhibited the highest concentration in the AFG, whereas the growth factors evaluated in the Serafini study exhibited

much lower concentrations in our samples. The difference may be due to the mode of analysis used in each study.

Ehrenfest et. al. quantified release of key growth factors and coagulation matrix glycoprotein from PRF membranes *in vitro*.[11] They evaluated the release of growth factors with ELISA at 20 min, 1 hour, 4 hours, 1 day, 3 day, 5 day and 7 day. The authors found that TGFb-1, PDGF-AB, TSP-1 were quickly released in 24 hours with slower release up to day 5, whereas VEGF showed rapid release in the first 4 hours, followed by a slower release after this time. In comparison, PDGF-AA, PDGF-AB, PDGF-BB, IGFBP-2, IGFBP-6, MCSF R and EGF exhibited the highest concentration in PRF samples in our study and showed a trend toward increases at 1.5 hours followed by a decrease at 3 hours. MCSF R showed similar levels throughout the 3 hours. Although it is difficult to compare the two studies due to differences in time of evaluation, it can be postulated that an initial rapid release of growth factors may have a critical contribution to the healing potential of PRF in the early stages.

It is also interesting to note that from both PRF and AFG, IGFBP-2 and IGFBP-6, MCSF R represented one of the highest concentrations of growth factors. PDGF-AA, PDGF-AB, PDGF-BB and EGF were present in much higher concentration in PRF than AFG, whereas AFG contained higher concentrations of IGFBP-1, IGFBP-2, IGFBP-4 and IGFBP-6.

Platelet-derived growth factor (PDGF) is a multiple mitogen with various isoforms (AA, AB, BB) that is involved in angiogenesis and proliferation of mesenchymal cells like fibroblasts and osteoblasts, and chemotaxis of mesenchymal cells and inflammatory cells.[18; 19] Studies have also shown PDGF-AA to be a key component of osteoblast proliferation and PDGF-BB was shown to accelerate tendon healing in both *in vitro* and *in vivo* studies using a fibrin-based delivery device.[15; 30] Additionally, PDGF-BB in the product GEM21 has been widely used for periodontal

regeneration.[23; 24] Due to high concentration of PDGF isoforms contained within a fibrin network in PRF samples, PRF as a biomaterial represents an ideal delivery vehicle to promote healing and proliferation of the surgical site.

Insulin-like growth factors (IGF) bind to insulin-like growth factor 1 receptor (IGF1R) to activate the IGF signaling pathway to inhibit apoptosis and promote cell proliferation and differentiation.[7] The half-life of unbound IGF is less than 10 minutes.[17] However, the half-life increases to 25 minutes and up to 16 hours when bound to insulin-like growth factor binding proteins (IGFBP).[17; 27] In vivo animal models have also shown that IGFBP-2 and IGFBP-4 greatly increase bone formation.[5; 22] Previous studies on PRF and AFG did not focus on IGFBP types but our results indicate that they are a large component of the growth factors released from the two biomaterials, and therefore, they warrant a greater consideration when using PRF and AFG.

Lastly, macrophage colony-stimulating factor receptor (MCSF R) and MCSF have not been commonly evaluated in studies of PRF and AFG. MCSF and its receptor have been shown to promote hematopoiesis, through macrophage production of G-CSF, IFN, TNF and IL-1, and major growth factors for osteoclasts through proliferation and differentiation of osteoclast progenitors.[16] Additionally, MCSF was found to promote growth of murine primary stromal initiating cells (SICs) that support proliferation of B and myeloid cells.[6] MCSF also activates the anti-bacterial and anti-fungal activities of macrophages in vitro and in vivo through increased phagocytic capacity, production of reactive oxygen intermediates, and increased killing capacity of micro-organisms.[28] Our results indicate that the MCSF R represents one of the higher concentrations of growth factors in both PRF and AFG and may contribute to the biomaterials potentially providing greater protection from infection after surgical procedures. A recent study

also showed that MCSF from a glioblastoma was able to induce IGFBP-1 to activate angiogenesis.[25] Although many growth factors in the PRF and AFG setting are often evaluated in isolation, it may be prudent to evaluate potential interactions and synergistic or antagonistic effects in future studies.

This study represents an early pilot study evaluating growth factors not previously evaluated for PRF and AFG. Due to the small sample size, statistically significant differences could not be clearly discerned. In addition, due to the method of evaluating the concentration of each growth factor with an antibody array rather than a growth factor specific ELISA, the concentration of each growth factor could only be compared relatively without knowing the exact concentration. However, this study discovered new growth factors not commonly evaluated for PRF and AFG and a potential trend showing changes in the concentrations of growth factors in the first 3 hours of blood draw.

5. Conclusion

This study was the first of its kind to evaluate a large array of growth factors not usually evaluated in studies of PRF and AFG. Within the limitations of the study, these data revealed an overall trend towards increasing growth factor concentrations at 1.5 hour followed by a decrease at 3 hours. In addition, the data revealed a distinct difference in growth factor composition between PRF and AFG. Further research is warranted with larger sample sizes to evaluate if there is a significant difference in growth factor concentration over time and between PRF and AFG. In addition, a further investigation is warranted with regards to the IGFBP growth factors and MCSF R and their contributions the beneficial effects of PRF and AFG.

References

[1]Ali, S., Bakry, S. A., & Abd-Elhakam, H. (2015). Platelet-Rich Fibrin in Maxillary Sinus Augmentation: A Systematic Review. *J Oral Implantol, 41*(6), 746-753. doi:10.1563/aaid-joi-D-14-00167

[2]Choukroun, J. (2000). Une opportunité en paro-implantologie: le PRF. *Implantodontie, 42*, 55–62.

[3]Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., . . . Dohan, D. M. (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 101*(3), e56-60. doi:10.1016/j.tripleo.2005.07.011

[4]Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., . . . Dohan, D. M. (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 101*(3), 299-303. doi:10.1016/j.tripleo.2005.07.012

[5]Conover, C. A., Johnstone, E. W., Turner, R. T., Evans, G. L., John Ballard, F. J., Doran, P. M., & Khosla, S. (2002). Subcutaneous administration of insulin-like growth factor (IGF)-II/IGF binding protein-2 complex stimulates bone formation and prevents loss of bone mineral density in a rat model of disuse osteoporosis. *Growth Horm IGF Res, 12*(3), 178-183. doi:10.1016/s1096-6374(02)00044-8

[6]Deryugina, E. I., Ratnikov, B. I., Bourdon, M. A., Gilmore, G. L., Shadduck, R. K., & Muller-Sieburg, C. E. (1995). Identification of a growth factor for primary murine stroma as macrophage colony-stimulating factor. *Blood, 86*(7), 2568-2578.

[7]Ding, H., & Wu, T. (2018). Insulin-Like Growth Factor Binding Proteins in Autoimmune Diseases. *Front Endocrinol (Lausanne), 9*, 499. doi:10.3389/fendo.2018.00499

[8]Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J., & Gogly, B. (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 101*(3), e37-44. doi:10.1016/j.tripleo.2005.07.008

[9]Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J., & Gogly, B. (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 101*(3), e45-50. doi:10.1016/j.tripleo.2005.07.009

[10]Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J., & Gogly, B. (2006). 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, 101*(3), e51-55. doi:10.1016/j.tripleo.2005.07.010

[11]Dohan Ehrenfest, D. M., de Peppo, G. M., Doglioli, P., & Sammartino, G. (2009). Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. *Growth Factors, 27*(1), 63-69. doi:10.1080/08977190802636713

[12]Dohan Ehrenfest, D. M., Doglioli, P., de Peppo, G. M., Del Corso, M., & Charrier, J. B. (2010). Choukroun's platelet-rich fibrin (PRF) stimulates in vitro proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way. *Arch Oral Biol, 55*(3), 185-194. doi:10.1016/j.archoralbio.2010.01.004

[13]Dohan Ehrenfest, D. M., Rasmusson, L., & Albrektsson, T. (2009). Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol, 27*(3), 158-167. doi:10.1016/j.tibtech.2008.11.009

[14]Dong-Seok Sohn, B. H., Jin Kim,Eric Park, Charles C. Park. (2015). Utilization of autologous concentrated growth factors (CGF) enriched bone graft Matrix (sticky bone) and CGF enriched fibrin membrane in implant dentistry. *The Journal of Implant & Advanced Clinical Dentistry, 7*, 11-29.

[15]Evrova, O., & Buschmann, J. (2017). In vitro and in vivo effects of PDGF-BB delivery strategies on tendon healing: a review. *Eur Cell Mater, 34*, 15-39. doi:10.22203/eCM.v034a02

[16]Fixe, P., & Praloran, V. (1998). M-CSF: haematopoietic growth factor or inflammatory cytokine? *Cytokine, 10*(1), 32-37. doi:10.1006/cyto.1997.0249

[17]Guler, H. P., Zapf, J., Schmid, C., & Froesch, E. R. (1989). Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinol (Copenh), 121*(6), 753-758. doi:10.1530/acta.0.1210753

[18]Hannink, M., & Donoghue, D. J. (1989). Structure and function of platelet-derived growth factor (PDGF) and related proteins. *Biochim Biophys Acta, 989*(1), 1-10. doi:10.1016/0304-419x(89)90031-0

[19]Heldin, C. H. (1992). Structural and functional studies on platelet-derived growth factor. *EMBO J, 11*(12), 4251-4259.

[20]Isobe, K., Suzuki, M., Watanabe, T., Kitamura, Y., Suzuki, T., Kawabata, H., . . . Kawase, T. (2017). Platelet-rich fibrin prepared from stored whole-blood samples. *Int J Implant Dent, 3*(1), 6. doi:10.1186/s40729-017-0068-4

[21]Lei, L., Yu, Y., Han, J., Shi, D., Sun, W., Zhang, D., & Chen, L. (2020). Quantification of growth factors in advanced platelet-rich fibrin and concentrated growth factors and their clinical efficacy as adjunctive to the GTR procedure in periodontal intrabony defects. *J Periodontol, 91*(4), 462-472. doi:10.1002/JPER.19-0290

[22]Miyakoshi, N., Qin, X., Kasukawa, Y., Richman, C., Srivastava, A. K., Baylink, D. J., & Mohan, S. (2001). Systemic administration of insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) increases bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism. *Endocrinology, 142*(6), 2641-2648. doi:10.1210/endo.142.6.8192

[23]Nevins, M., Giannobile, W. V., McGuire, M. K., Kao, R. T., Mellonig, J. T., Hinrichs, J. E., . . . Lynch, S. E. (2005). Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. *J Periodontol, 76*(12), 2205-2215. doi:10.1902/jop.2005.76.12.2205

[24]Nevins, M., Kao, R. T., McGuire, M. K., McClain, P. K., Hinrichs, J. E., McAllister, B. S., . . . Giannobile, W. V. (2013). Platelet-derived growth factor promotes periodontal regeneration in localized osseous defects: 36-month extension results from a randomized, controlled, double-masked clinical trial. *J Periodontol, 84*(4), 456-464. doi:10.1902/jop.2012.120141

[25]Nijaguna, M. B., Patil, V., Urbach, S., Shwetha, S. D., Sravani, K., Hegde, A. S., . . . Somasundaram, K. (2015). Glioblastoma-derived Macrophage Colony-stimulating Factor (MCSF) Induces Microglial Release of Insulin-like Growth Factor-binding Protein 1 (IGFBP1) to Promote Angiogenesis. *J Biol Chem, 290*(38), 23401-23415. doi:10.1074/jbc.M115.664037

[26]Öncü, E. (2017). The Use of Platelet-Rich Fibrin Versus Subepithelial Connective Tissue Graft in Treatment of Multiple Gingival Recessions: A Randomized Clinical Trial. *The International Journal of Periodontics & Restorative Dentistry, 37*(2), 265–271. doi:10.11607/prd.2741

[27]Rajaram, S., Baylink, D. J., & Mohan, S. (1997). Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. *Endocr Rev, 18*(6), 801-831. doi:10.1210/edrv.18.6.0321

[28]Ralph, P., & Sampson-Johannes, A. (1990). Macrophage growth and stimulating factor, M-CSF. *Prog Clin Biol Res, 338*, 43-63.

[29]Serafini, G., Lopreiato, M., Lollobrigida, M., Lamazza, L., Mazzucchi, G., Fortunato, L., . . . De Biase, A. (2020). Platelet Rich Fibrin (PRF) and Its Related Products: Biomolecular Characterization of the Liquid Fibrinogen. *J Clin Med, 9*(4). doi:10.3390/jcm9041099

[30]Yang, D., Chen, J., Jing, Z., & Jin, D. (2000). Platelet-derived growth factor (PDGF)-AA: a self-imposed cytokine in the proliferation of human fetal osteoblasts. *Cytokine, 12*(8), 1271-1274. doi:10.1006/cyto.2000.0707

[31]You, T. M., Choi, B. H., Zhu, S. J., Jung, J. H., Lee, S. H., Huh, J. Y., . . . Li, J. (2007). Platelet-enriched fibrin glue and platelet-rich plasma in the repair of bone defects adjacent to titanium dental implants. *Int J Oral Maxillofac Implants, 22*(3), 417-422.

Figures

Each antibody is spotted in duplicate vertically

	A	B	C	D	E	F	G	H	I	J	K	L
1 2	POS	POS	NEG	NEG	AR	bFGF	beta-NGF	EGF	EGFR	FGF-4	FGF-6	FGF-7 (KGF)
3 4	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-1	IGF-1 R
5 6	IGF-2	M-CSF	M-CSF R	NT-3	NT-4	PDGF R alpha	PDGF R beta	PDGF-AA	PDGF-AB	PDGF-BB	PLGF	SCF
7 8	SCF R (CD117)	TGF alpha	TGF beta 1	TGF beta 2	TGF beta 3	VEGF-A	VEGFR2	VEGFR3	VEGF-D	BLANK	BLANK	POS

Figure 3. Human Growth Factor Array. RayBio® Human Growth Factor Antibody Array C1 with 41 human growth factors was used for the study.

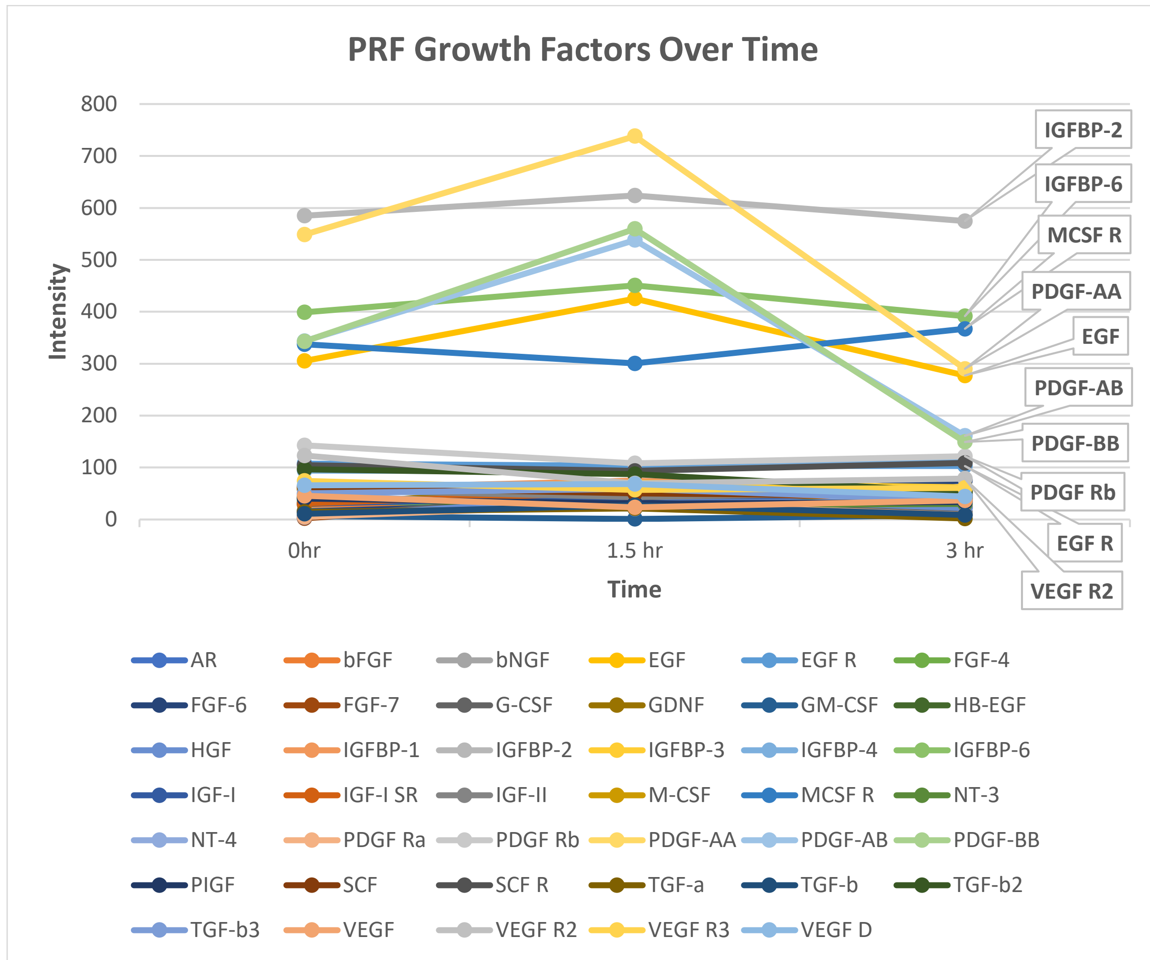

Figure 4. 41 Growth Factors in PRF at 0 Hr, 1.5 Hr, 3 Hrs. Aggregate graph of all growth factors evaluated in PRF over time. Intensity values indicate average of the three subjects. Growth factors of highest concentration are denoted on the right.

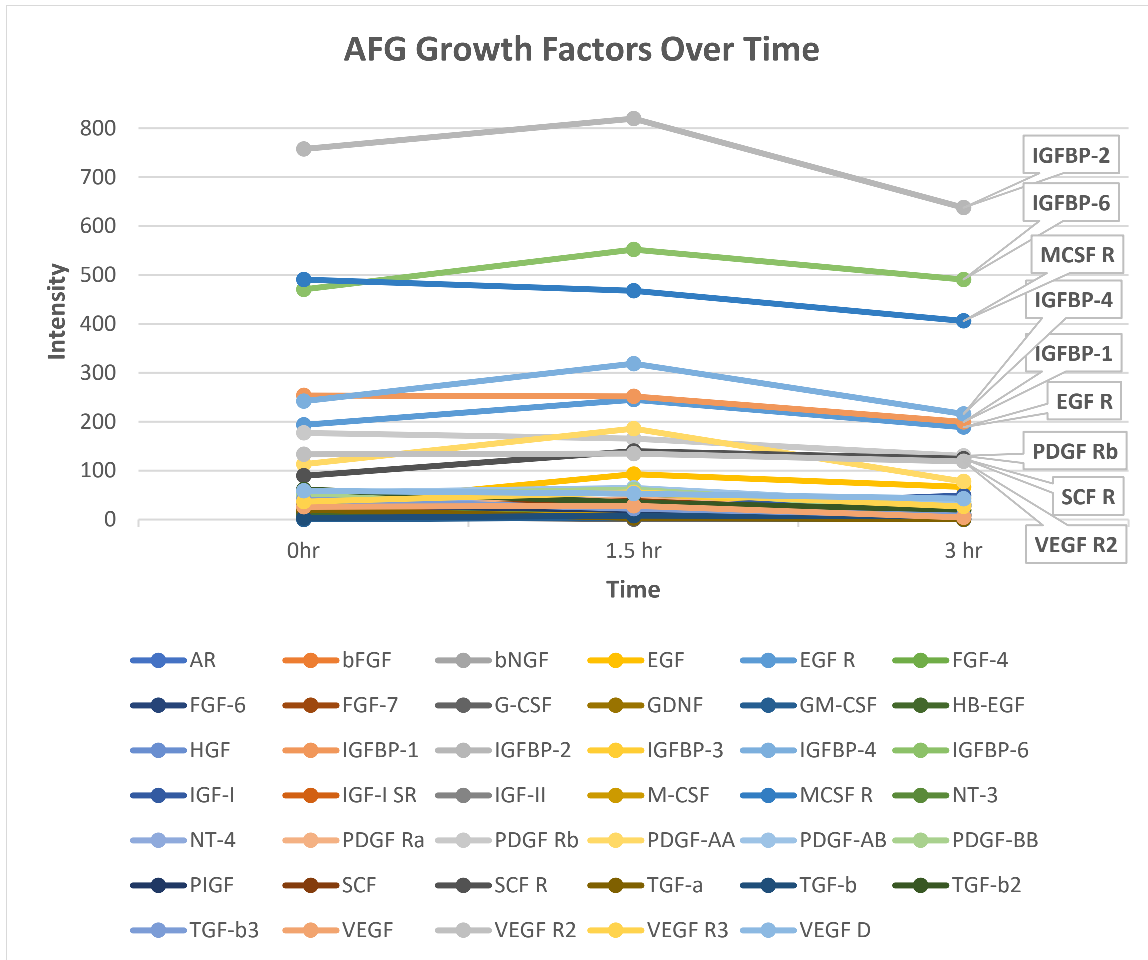

Figure 5. 41 Growth Factors in AFG at 0 Hr, 1.5 Hr, 3 Hrs. Aggregate graph of all growth factors evaluated in PRF over time. Intensity values indicate average of the three subjects. Growth factors of highest concentration are denoted on the right.

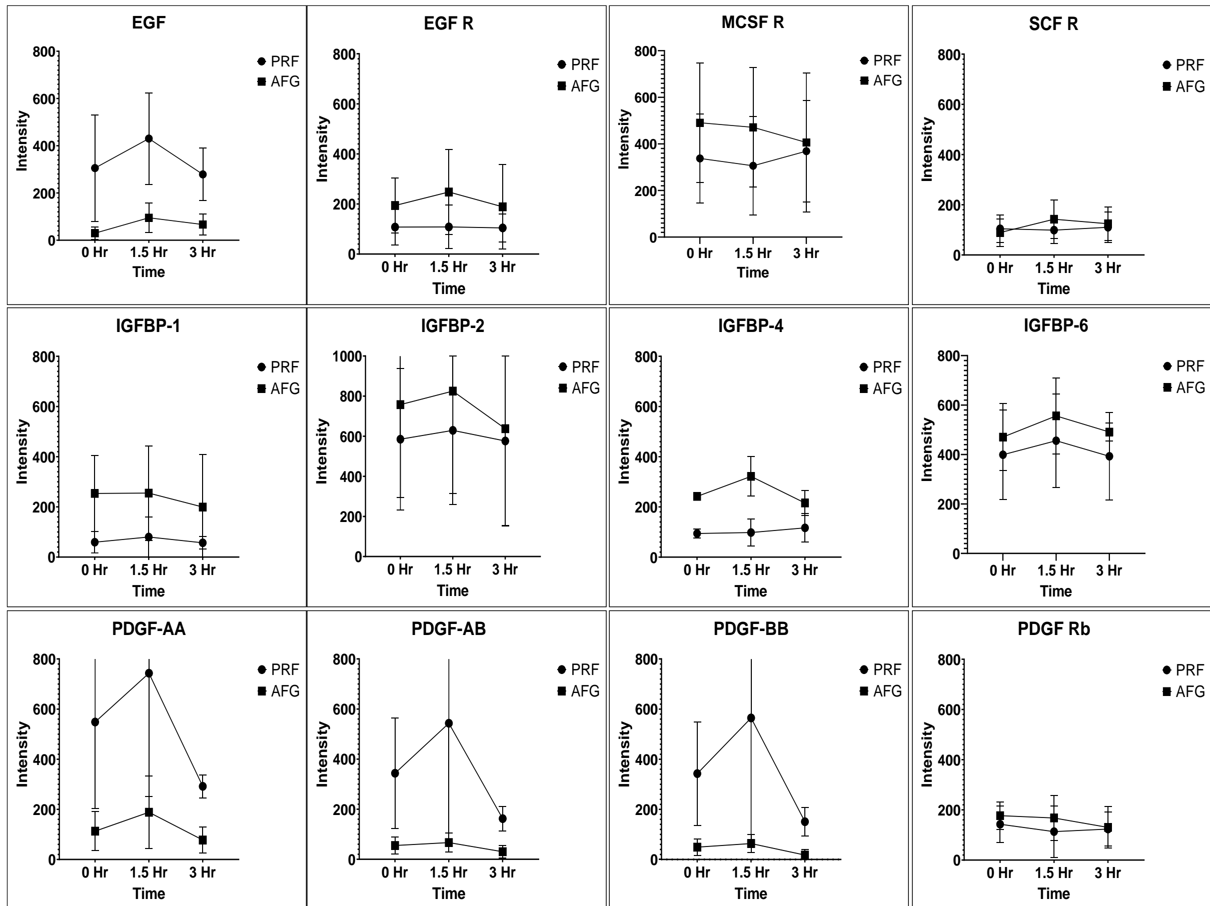

Figure 6. Individual Graphs for Top Growth Factors. Individual graphs of growth factors comparing concentration in PRF and AFG. Error bars indicate standard deviation. Higher concentration of IGFBP-1,2,4,6 for AFG and higher concentration of PDGF-AA, AB, BB for PRF.

Tables

Table 1. Growth Factor Over Time. **Indicates statistical significance*

Growth Factor	Comparison	Time 1	Time 2	Q Value	Significance
EGF	PRF 0 Hr vs 1.5 Hr	305.4	430.4	0.0854	No
	PRF 1.5 Hr vs 3 Hr*	430.4	278.7	0.0494	**Yes**
	PRF 0 Hr vs 3 Hr	305.4	278.7	0.3685	No
	AFG 0 Hr vs 1.5 Hr	29.96	94.88	0.2584	No
	AFG 1.5 Hr vs 3 Hr	94.88	66.46	0.3685	No
	AFG 0 Hr vs 3 Hr	29.96	66.46	0.3685	No
IGFBP-4	PRF 0 Hr vs 1.5 Hr	93.98	98.06	0.2491	No
	PRF 1.5 Hr vs 3 Hr	98.06	116.7	0.1583	No
	PRF 0 Hr vs 3 Hr	93.98	116.7	0.1428	No
	AFG 0 Hr vs 1.5 Hr*	241.7	321.7	0.004	**Yes**
	AFG 1.5 Hr vs 3 Hr*	321.7	215.8	0.0014	**Yes**
	AFG 0 Hr vs 3 Hr	241.7	215.8	0.1428	No
PDGF-AA	PRF 0 Hr vs 1.5 Hr	548.4	743.3	0.2083	No
	PRF 1.5 Hr vs 3 Hr*	743.3	291.6	0.0147	**Yes**
	PRF 0 Hr vs 3 Hr	548.4	291.6	0.1225	No
	AFG 0 Hr vs 1.5 Hr	113.1	188.3	0.4227	No
	AFG 1.5 Hr vs 3 Hr	188.3	77.66	0.3796	No
	AFG 0 Hr vs 3 Hr	113.1	77.66	0.5273	No
PDGF-AB	PRF 0 Hr vs 1.5 Hr	343.7	543.2	0.1901	No
	PRF 1.5 Hr vs 3 Hr*	543.2	162.5	0.0178	**Yes**
	PRF 0 Hr vs 3 Hr	343.7	162.5	0.4308	No
	AFG 0 Hr vs 1.5 Hr	55.1	66.74	0.7149	No
	AFG 1.5 Hr vs 3 Hr	66.74	30.22	0.7118	No
	AFG 0 Hr vs 3 Hr	55.1	30.22	0.7126	No
PDGF-BB	PRF 0 Hr vs 1.5 Hr	342.7	564.8	0.1425	No
	PRF 1.5 Hr vs 3 Hr*	564.8	150.7	0.0102	**Yes**
	PRF 0 Hr vs 3 Hr	342.7	150.7	0.1938	No
	AFG 0 Hr vs 1.5 Hr	49.15	63.56	0.7026	No
	AFG 1.5 Hr vs 3 Hr	63.56	17.76	0.6708	No
	AFG 0 Hr vs 3 Hr	49.15	17.76	0.6853	No

Table 2. Growth Factor Comparison PRF vs AFG **Indicates statistical significance*

Growth Factor	Time	PRF	AFG	Q Value	Significance
EGF	**0 Hr***	305.4	29.96	0.0032	**Yes**
	1.5 Hr*	430.4	94.88	0.0008	**Yes**
	3 Hr*	278.7	66.46	0.0222	**Yes**
IGFBP-4	**0 Hr***	93.98	241.7	<0.0001	**Yes**
	1.5 Hr*	98.06	321.7	<0.0001	**Yes**
	3 Hr*	116.7	215.8	0.0021	**Yes**
PDGF-AA	**0 Hr***	548.4	113.1	0.0153	**Yes**
	1.5 Hr*	743.3	188.3	0.0035	**Yes**
	3 Hr	291.6	77.66	0.2083	No
PDGF-AB	0 Hr	343.7	55.1	0.0657	No
	1.5 Hr*	543.2	66.74	0.005	**Yes**
	3 Hr	162.5	30.22	0.4215	No
PDGF-BB	0 Hr	342.7	49.15	0.0646	No
	1.5 Hr*	564.8	63.56	0.003	**Yes**
	3 Hr	150.7	17.76	0.4254	No

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