

Characterization of Growth Factors in Platelet Rich Plasma

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Introduction

Growth factors released from activated platelets initiate and modulate wound healing in both soft and hard tissues. A recent strategy to promote the wound-healing cascade is to prepare an autologous platelet concentrate suspended in plasma, also known as platelet-rich plasma (PRP). This PRP is then administered to sites where soft and hard tissue healing are necessary. There have been numerous clinical studies demonstrating the efficacy of PRP use in a variety of applications. The growth factors released from the alpha granules of the platelets are the driving force behind the up-regulation of healing that has been documented. A study was performed to monitor the growth factor release from a clot during *in vitro* plasmin-mediated clot clearance. It was seen that release characteristics varied significantly for the different growth factors assayed in terms of percent of maximum concentration released during initial degranulation and the time until 80% of the maximum concentration was released.

Materials and Methods

Processing of PRP: The GPS II Platelet Concentrate Separation Kit (Cell Factor Technologies, Warsaw IN) was used to produce 6cc of PRP from a 55cc blood draw. This process involved a single 15 minute centrifugation cycle and in total took <20 minutes

Clot Retraction: For each donor, fourteen 200 μ l PRP samples were activated using 20 μ l of 1000 U/ml bovine thrombin in 1m CaCl₂. 1ml of 0.01 U/ml human plasmin in normosaline was added to each PRP sample. At 0, 0.5, 1, 2, 4, 8, and 20 hours, 2 of the fourteen samples were randomly selected for each donor and the entire supernatant was aspirated and immediately frozen.

Growth Factor Assays: ELISA assays were used to determine the growth factor concentrations in the collected samples. Each sample was run in duplicate and the data from the two samples taken at each time point were averaged to calculate the concentration of growth factor released at that time point

Results

Hematology: An average yield of 6.0 \pm 0.40ml of PRP for the five donors. The average whole blood platelet counts, PRP platelet counts and platelet concentration factors were 181 \pm 29.2 $\times 10^3$ cells/ml, 1451 \pm 223 $\times 10^3$ cells/ml, and 8.02 \pm 0.35 respectively.

Elution at time of degranulation: Table 1 shows the amount of each growth factor that was eluted at initial degranulation in picograms per million platelets and percentage of maximum concentration.

Growth Factor	Amount (pg/10 ⁶ platelets)	S.D.	% of Max
EGF	7.59	2.83	13.21
bFGF	2.73	0.42	35.58
PDGF-BB	11.65	10.42	15.02
TGF- β_1	633	300	49.17
VEGF	13.02	7.21	25.37

Table I-Shows the amount of each growth factor detected in the supernatant at initial degranulation (t=0 hours)

Time at 80% elution Table II shows the time point that the concentration of each growth factor exceeded 80% of the maximum concentration detected. The table also shows the exact percentage of the maximum concentration at the time point where the 80-% threshold was exceeded.

Growth Factor	Timepoint (hours)	% of Max.
EGF	8	85.6
bFGF	2	82.3
PDGF-BB	4	82.4
TGF- β_1	2	80.7
VEGF	4	80.4

Table II-Shows the timepoint at which the 80% of maximum concentration released threshold was passed and the actual percentage of the maximum concentration released at that time point

Discussion

The data obtained during the experiments showed that platelet derived growth factors are released from a blood clot at differing rates TGF- β_1 and bFGF were seen to elute most strongly upon initial degranulation. Of the five growth factors assayed these two are most commonly linked with the early stages of wound repair including the inflammatory stage and the migration and proliferation of fibroblasts in the wound bed. It was also seen that EGF was retained in the clot longer than all of the other growth factors assayed. Epithelial migration and proliferation, which is regulated in part via EGF release is associated with later stage wound repair. Analysis of this data leads to a compelling insight of the synergistic actions of platelet-derived growth factors during wound repair. Future iterations of this experiment will be focused on further elucidating these synergisms