

# Characterization of Autologous Growth Factors in Cascade® Platelet-Rich Fibrin Matrix (PRFM)

Richard J. Carroll, PhD<sup>1</sup>, Steven P. Arnoczky, DVM<sup>2</sup>, Susan Graham MS, MT (ASCP), SH<sup>3</sup> and Sean M. O'Connell, PhD<sup>1&4</sup>

## INTRODUCTION

Platelet-derived growth factors released from activated platelets following injury initiate and drive the early (bFGF, PDGF, IGF) and later (EGF, VEGF, TGF- $\beta$ , IGF) stages of healing in bone and soft tissue. A variety of methods have been developed to employ these growth factors in the form of an autologous platelet-rich plasma (PRP) to accelerate the healing process. This PRP is usually produced by making a platelet concentrate from the patient's blood after separation from the other blood component (RBC, WBC, Fibrin). The PRP is then mixed with CaCl<sub>2</sub> and excess bovine thrombin to create a stable clot.

The Cascade® PRFM employs a novel strategy—concentrating both platelets and fibrin in a dense fibrin matrix without the use of bovine thrombin. The lack of excess exogenous thrombin ensures platelet integrity during the production process and avoids premature growth factor release associated with bovine thrombin activation and degranulation. While numerous studies have documented the growth factors released from the platelet granules and their efficacy in stimulating rapid healing in bone and soft tissue, this study (Study I) was designed to measure the number, amount and time-course of specific in vitro growth factors released from PRFM made with the Cascade system.

In a second study (Study II), the kinetics of platelet growth factor expression over the course of seven days following blood draw and PRFM production was examined in a “wash-out” experiment. Given the variability of growth factor stability in aqueous saline solution, this time-course study employs a “wash-out” in order to assess specific growth factors produced at each time point without the contribution of growth factors present before that time point (carry-over).

## MATERIALS AND METHODS (STUDY I): A SEVEN-DAY IN VITRO TIME-COURSE

**Processing of the PRFM:** The Cascade system (Cascade Medical Enterprises, Wayne, NJ) was used to produce approximately 2cc of PRFM from a 9cc blood draw. The process involves a six-minute, 1100g spin in the separator tube to obtain a PRP, transfer to a CaCl<sub>2</sub> containing clot tube followed by a second 15-minute centrifugation at 1450g to produce the PRFM and residual serum.

**Design:** Following centrifugation, replicate PRFM samples were macerated with a scalpel and placed into polypropylene tubes together with normal saline in three groups. These groups of PRFM samples were incubated at room temperature for one hour, 24 hours and 168 hours respectively. All samples were harvested at the appropriate time points and stored at -78°C for later Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis. Ten samples from a single donor were employed for this study.

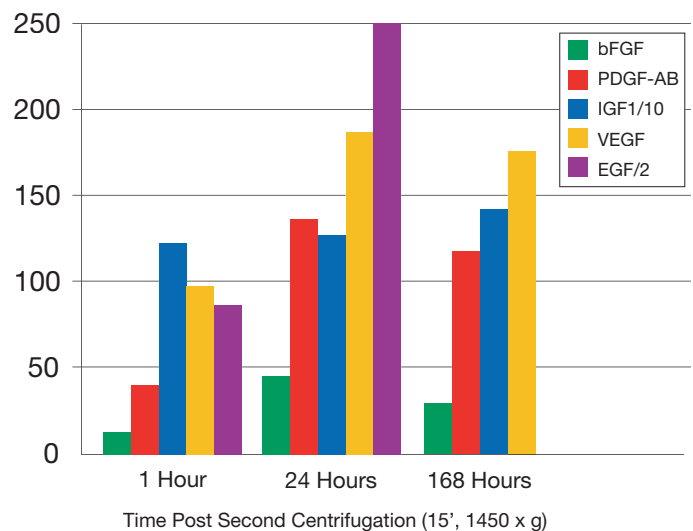
**Growth Factor Assays:** Specific growth factor levels released from each gel were measured using the ELISA system (R&D Systems, Minn., MN). Each sample was run in duplicate and average growth factor concentration for each time point that was calculated. The growth factors studied were insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor-AB (PDGF-AB) and vascular endothelial growth factor (VEGF).

## RESULTS (STUDY I)

**Time-Course of Growth Factor Release:** The concentration of specific growth factors released at each time in picograms/mL sample is given in the bar chart below.

### PRFM STUDY I: SUMMARY OF RESULTS

Human Growth Factor Expression Over Time – Non “Wash-Out”



*Note: IGF and EGF must be multiplied by ten and two respectively to get actual GF levels. EGF at 168 hours was not done.*

## DISCUSSION (STUDY I)

These results show that growth factors (GFs) are measurable in vitro when released from the PRFM over time. Significant GF levels were measured as early as one hour and out to 168 hours. The study also showed that the kinetics and total concentration released varied between different GFs. Some factors produced sustained high levels from one to 168 hours (IGF-1), while others started low and reached high levels by 24 hours and maintained them out to seven days (PDGF, VEGF) or went from low to high and then dropped to lower levels (bFGF). Absolute GF levels varied from a low of 10.5 pg/mL (bFGF, one hour) to a high of 1425 pg/mL (IGF-1, 168 hours). These results demonstrate that the platelets within the PRFM remain functionally viable throughout the Cascade process and suggest a continual release out to seven days.

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## MATERIALS AND METHODS (STUDY II): A SEVEN-DAY IN VITRO "WASH-OUT" TIME-COURSE

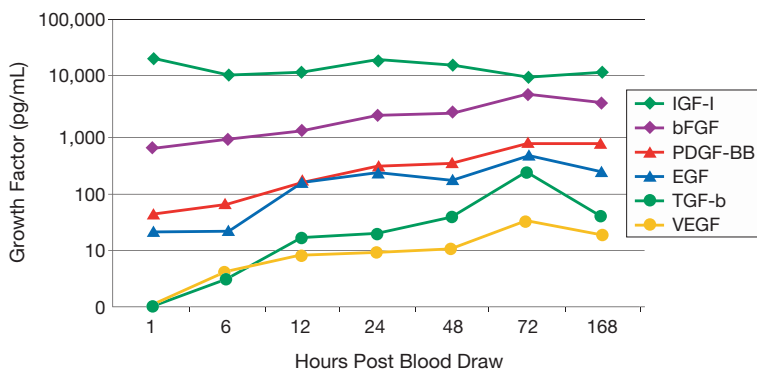
**Design:** Two mLs of sterile saline solution were added to each PRFM and allowed to incubate for varying amounts of time at room temperature. At each time point, the 2.0 mL incubation solution was removed and stored at -78°C for later ELISA analysis, while a fresh 2.0 mL saline aliquot was added to the gel and allowed to incubate until the next time point. This process was repeated at each time point until the saline in tube #8 was removed from the PRFM at 168 hours, the volume measured and placed in cryovials and stored at -78°C until ELISA analysis. In this manner, only the growth factors released since the previous time point would be measured (the "wash-out"). Eight blood samples from a single donor were employed for this study.

**Processing of the PRFM:** The PRFM was processed as described in Study I.

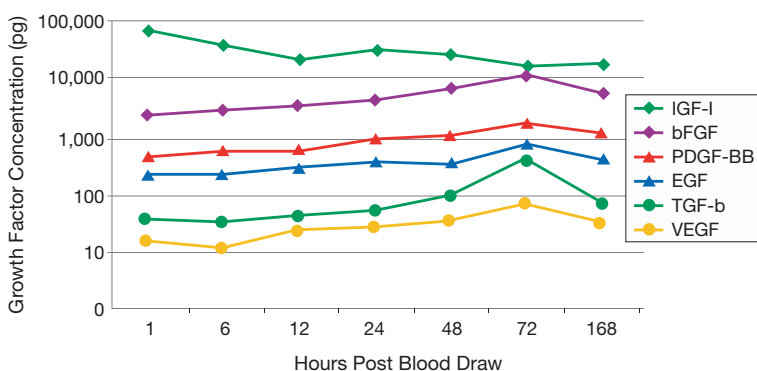
**Growth Factor Assays:** The specific growth factors were assayed as described in Study I with the addition that TGF- $\beta$  levels were also measured.

## PRFM STUDY II: SUMMARY OF RESULTS

### Platelet-Derived Growth Factor Concentration at Each Time Point



### Cumulative Platelet-Derived Growth Factor Production Over Seven Days



## DISCUSSION

In addition to confirming the findings of Study I that all of the GFs examined were present throughout the study and that the kinetics and total concentration produced varied between different GFs, Study II showed that the platelets within the PRFM remain functionally viable throughout the Cascade process and continually released all GFs within the PRFM out to seven days in vitro at room temperature. Further, the production of five out of the six GFs examined exhibited an increasing rate of release out to 72 hours post blood draw. The exception is IGF-I which maintained a relatively constant level. However, IGF-I exhibited the highest levels by ten to 1000 times over the other GFs examined.

Finally, comparison of the "wash-out" time-course with the cumulative time-course demonstrated a 5X to 10X increase for each GF in the cumulative study. This finding suggests that the stability of the secreted GFs in the PRFM is greater than what would be predicted based on in vitro studies of GF stability in aqueous solution and that these GFs would be available to the cells and tissue of the wound site for prolonged periods ( $\geq$  seven days) during the healing process (Marx RE et al., 2001). Further studies are needed to confirm this observation.

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