INTRODUCTION:
Platelet-rich plasma (PRP) contains over 30 kinds of growth factors (GF) related to tissue healing in the α-granules including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), basic fibroblastic growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), IGF-1, and connective tissue growth factor (CTGF). Consensus about PRP production and characterization should be a prerequisite for clinical use. However, various investigators produce different PRPs via their own methods, making the interpretation of the results more difficult. The purpose of the study was to evaluate the kinetics of GF release in PRP, which had been produced consistently through plateletpheresis system for eliminating all possible variables, including number and activation status of platelets and contamination of RBC and WBC. In addition, for closer simulation of actual in vivo situation, the authors used wash-out method for the measurement of kinetics of GF release.

MATERIALS AND METHODS:
Preparation of leukodepleted PRP (n=3)
Leukodepleted platelet concentrates were prepared from a plateletpheresis system using Cobe LRS Turbo fluidized particle bed separation (Caridian BCT; Lakewood, CO, USA).
Measurement of GF concentrations
Platelets (PRP group) were diluted at 1 x 10⁷ cells/µL. Thrombin and calcium gluconate (Ca-G) were added to PPP and PRP samples at the following concentration:
- (i) PPP with 104.22mg/ml of Ca-G
- (ii) PRP (non-activation)
- (iii) PRP with 104.22mg/ml of Ca-G
- (iv) PRP with 166.67U/ml thrombin and 104.22mg/ml of Ca-G
Each group was incubated at 37°C for 10min. 1.5ml of sterile saline solution were added to each group and incubated for 30min, 1hr, 3hr, 6hr, 12hr, 24hr, 72hr at 37°C in a 5% CO₂ incubator. At each time point, the 1.5mL incubation solution was removed and stored at -78°C for ELISA assay, while a fresh 1.5mL saline solution was added to each group and allowed to incubate until the next time point (wash-out). Specific growth factor levels released from each group were measured using the ELISA kit (IGF-I, EGF, VEGF, TGF-β1 basic-FGF, CTGF).

RESULTS:

DISCUSSION:
While there have been many studies about the GF concentrations in PRP, majority of them had drawbacks for interpretation or comparing the results, because PRP production process was different with each other and characterization of PRP could not be performed more clearly. In addition, most of reports used non-wash-out measurement, which would not represent in vivo circumstance. In this study, we controlled all noticeable variables through leukodepleted plateletpheresis system and by using consistent platelet concentrations, 1 x 10⁷ cells/µL in all experiments. And to simulate the real situation, we adopted wash-out method for measurement of GF kinetics.

Although the kinetics of six studied GF from inactivated PRP were different each other, generally each could be grouped into one of three types. Initial surge group, e.g. TGF-β1, IGF-1, and VEGF; delayed surge group, bFGF and CTGF; finally later surge group, EGF. When activated with calcium and thrombin, the kinetics of GF release changed, especially at the early time points, that is, during less than 12 hours.

REFERENCE: