Introduction
During the past years, application of PRP has become very popular in oral implantology. Local enrichment of growth factors in the wound by platelet concentration is regarded as an elegant method to stimulate tissue regeneration around implants and augmentation materials. Different protocols and devices for manufacturing of PRP “chair side” in the dental office are already commercially available (Anitua, PCCS-System, Curasan), although there are only few hard clinical data that support the effects claimed for application of PRP in tissue regeneration [1,2]. We therefore set out to investigate the effects of different PRP-fractions in an in vitro model system.

Materials
MC3T3-E1 preosteoblasts were precultured in 96-well microtiter plates in Alpha-MEM medium containing fetal calf serum (15%), glutamine (1%) and antibiotics (1%) for 24 hours. Subsequently, the cells were transferred to basal medium (without FCS) containing 5, 20 and 40% PRP for 24 hours. Controls were: a) cells with 5, 20 and 40% platelet poor plasma (PPP), b) cells with the coagulating agents of the PRP-preparation (CaCl₂, thrombin) and c) cells without additional supplements. Proliferation of the cells was assayed by incorporation of BrdU (5-bromo-2’-deoxyuridine) for 24h. PRP was prepared from the centrifugation supernatant of anticoagulated blood according to the Curasan method [3]. Coagulation of PRP-preparations was initiated by addition of 10 vol% of 5% CaCl₂ containing 1000U/ml thrombin. After one hour, the coagulated platelet concentrate was centrifuged with 18000g for 15 min. The supernatant containing the growth factors was added to the cell cultures.

Results & Discussion
Cell proliferation was enhanced by addition of 40% PRP supernatant to the cell cultures to 59% in comparison to cells in basal medium. A comparable stimulation, (mean 58%) was achieved by addition of platelet poor plasma (PPP). Under certain experimental conditions, however, addition of thrombin alone also caused stimulative effects up to 45% (Fig.1).

Conclusions
Clinical studies have claimed that the application of platelet concentrates to bone grafts result in an acceleration of wound healing and a higher bone density [1,2]. The beneficial effects of PRP shall be caused by growth factors released from the Alpha-granules of the platelet concentrates. Under our experimental conditions, stimulation of cell proliferation by PRP seemed to be due to thrombin and serum components of the PRP preparation rather than to growth factors released from the Alpha-granules of the thrombocytes.

Figure 1. Proliferation of MC3T3-E1-Preosteoblasts after 24h incubation in basal medium with 40% PRP. Columns represent means of three independent experiments with 95% confidence intervals.

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References