The effects of autogenous plasma and platelet-released growth factors in bone regeneration—In vitro and in vivo study—

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[Objectives]
The effects and differences of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in bone regeneration have been not indicated clearly. Our experimental studies have shown the favorable effects of platelet-poor plasma (PPP) in bone regeneration. This study evaluated the effect of autogenous plasma and platelet-released growth factors to bone formation.

[Methods]

**In vitro study**

Blood from healthy subjects was collected, after centrifugation PRP and PPP were taken. The concentrations of platelet-released growth factors in PRP, PPP, and whole blood were measured. The proliferation and the differentiation assay were examined using human bone marrow stromal cells.

**Preparation of PPP and PRP**

Human Blood 45mL +CPD 5ml → PPP (PRP/Ppoison+ 2%CaCl2/30μl)

Human Blood 4.5mL +CPD 0.5ml → PRP

CA-2Cl2 Anticoagulant

Centrifugation (400g, 10min)

**Preparation of PRF and PRP**

Blood 9ml +CPD 1ml → 37°C 2h incubation

350rpm, 10min

The lysates including growth factors were collected.

**ELISA (enzyme-linked immunosorbent assay)**

TGF-β1, VEGF, PDGF-AB concentration of PPP, PRP, Serum

**Preparation of human bone marrow stromal cell harvest and culture**

DMEM+10%FBS

Human MSCs were aspirated from femurs.

**In vivo study**

Twelve beagle dogs (male, 10.1-13.8kg)

**Preparation of PPP, PRP, and PRF**

Blood 9ml+CPD 1ml

Centrifugation (800g,10min)

The upper layer 2ml was collected as PPP.

**Preparation of PRF**

Blood 10ml without an anticoagulant

Centrifugation (700g,8min)

The PRF (the upper part of the tube) was obtained by centrifugation.

**Implantation of PPP, PRP, and PRF**

PPP, PRP, and PRF were implanted to each extraction socket with dehiscence in mandible.

**Radiographical analysis (micro-CT)**

Measurements were performed for three areas of each grafted site: the midline, 0.2 mm medial to the midline, and 0.2 mm lateral to the midline. The ROI was set as the size of 5 × 5 mm. The horizontal bone width at a 1.5 mm lower part was measured.

**Histological analysis**

Decalcified tissue specimens (HE stain) from each defect were analyzed histologically.

**Results**

**In vitro study**

The average concentrations of platelets, TGF-β1 and PDGF-AB in blood products were all increased in PRP and decreased in PPP. When different concentrations of platelet-released growth factors were added to the human MSC cultures, PRP showed a stimulative effect on proliferation. PRF showed a stimulative effect on osteoblastic differentiation of MSCs in a dose-dependent manner.

**Concentration of platelet-released growth factors**

**Effect of platelet-released growth factors on proliferation of hMSCs**

**Effect of growth factors on hMSCs**

**The effects of fibrin network**

Fibrinogen concentration: PRP < PPP

Strenght: PRP < PPP < PRF

**Space making**

PRP < PPP < PRF?

**Histological findings**

**Discussion**

In vitro studies, these results indicated that the soluble factors in PRP had a stimulative effect on proliferation of MSCs and an inhibitory effect on osteoblastic differentiation. In vivo studies, PRP and PRF did not promote bone formation in bone defect site that differentiated osteoblastic cells are few. Because, Growth factors can stimulate the proliferation of any cells, irrespective of whether or not the cells are involved in bone differentiation.

The reason why PRF promoted a greater amount of bone formation compared with PRP, even though both materials contained a rich concentration of growth factors in platelets, may be that the strength of PRF supported a more dense fibrin network.

**Conclusion**

This study showed that PPP is an effective material for the preservation of sockets with buccal dehiscence and PPP plays a significant role in the presence of fewer osteogenic cells. The fibrin network of PPP has played a role as space making for bone regeneration and would be stimulatory to bone formation. PPP has various advantages such as minimal errors among manufacturers and easy handling.