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

MARUKAWA Eriko*, HATAKEYAMA Ichiro, TAKAHASHI Yukinobu, OMURA Ken
Tokyo Medical and Dental University, Oral and Maxillofacial Surgery, Tokyo, Japan

【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.

	PPP	PRP	PRF
Platelet counts	low	high	high
platelet-released growth factors	low	high	high
Fibrin	Low, weak	Low, weak	High, strong
Anticoagulant	need	need	no need
Activation(+2%$CaCl_2$)	need	need	no need

Dohan et. al. ; Trends in Biotechnology 2009

【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 PRP and PPP

Human Blood 45ml +CPD 5ml → PRP, PPP(5ml)+ 2%$CaCl_2$(830μl)

Human Blood 4.5ml +CPD 0.5ml + 2%$CaCl_2$(830μl)

→ 37℃ 2h incubation 3500rpm, 10min

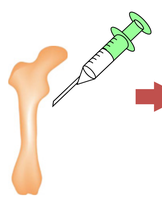
The lysates including growth factors were collected.

CPD: anticoagulant citrate phosphate dextrose

ELISA (enzyme-linked immunosorbent assay)

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

Human bone marrow stromal cell harvest and culture



Human MSCs were aspirated from femurs.

Passage1 → Passage2

DMEM+10%FBS

osteoblastic differentiation medium
- 100nM Dexamethasone
- 0.05mM Ascorbic acid phosphate
- 10mM β-glycerophosphate

- cell proliferation assay
- cell differentiation assay
- RT-PCR

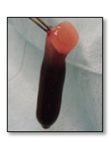
In vivo study

Twelve beagle dogs (male,10.1-13.8kg)

Preparation of PPP, PRP, and PRF

PPP
Blood 9ml+CPD1ml
↓
Centrifugation(800g,10min)
↓
The upper layer 2ml was collected as PPP.
↓
Activation (+2%$CaCl_2$)

PRF
Blood 10ml without an anticoagulant
↓
Centrifugation(400g,10min)
↓
PRF(the upper part of the tube)



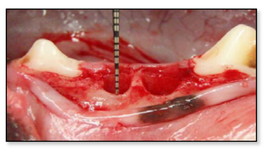
PRP
Blood 9ml+CPD1ml
↓
Centrifugation(700g,8min)
↓
Centrifugation(1600g,8min)
↓
2 ml of PRP was collected from the bottom layer
↓
Activation (+2%$CaCl_2$)

Scanning electron microscopy (SEM) observations

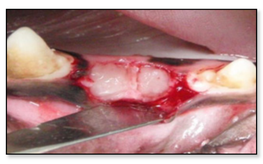
The morphology in each material of PPP, PRP, and PRF was analyzed using SEM.

Implantation of PPP, PRP, and PRF

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



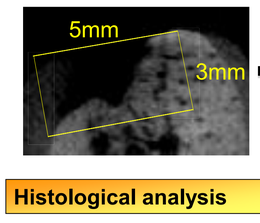
The third premolar was carefully extracted, and 3-mm dehiscence from the alveolar crest to the buccal wall was created in two roots of each extraction socket.



These materials were filled into each root of the extraction sockets. The extraction sockets of the control group were left unfilled.

Results were evaluated at 4 and 8 weeks after surgery (N=6).

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 × 3 mm. The horizontal bone width of a 1.5 mm lower part was measured.

The endpoint differences between the groups were analyzed using the Mann–Whitney U test ($p < 0.05$).

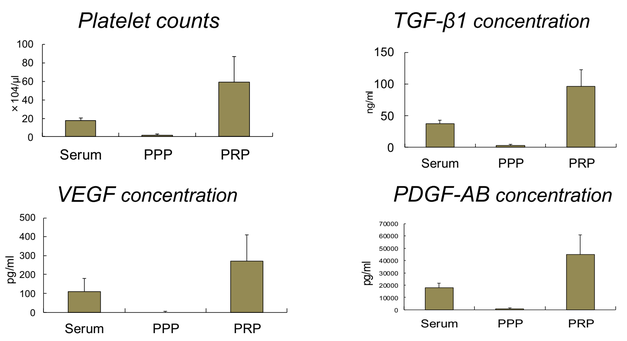
Histological analysis

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

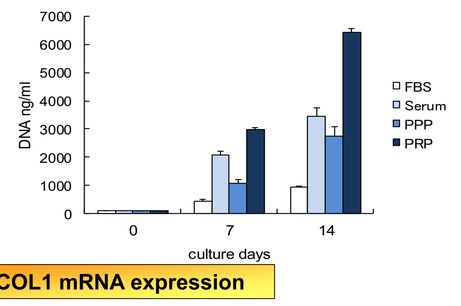
【Results】

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. Contrary to the effect of PRP on proliferation, PRP exhibited an inhibitory effect on osteoblastic differentiation of MSCs in a dose-dependent manner.

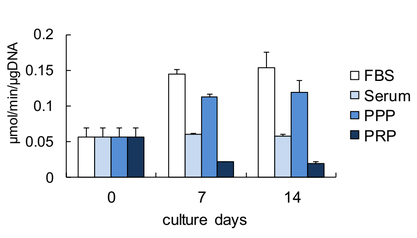
Concentration of platelet-released growth factors



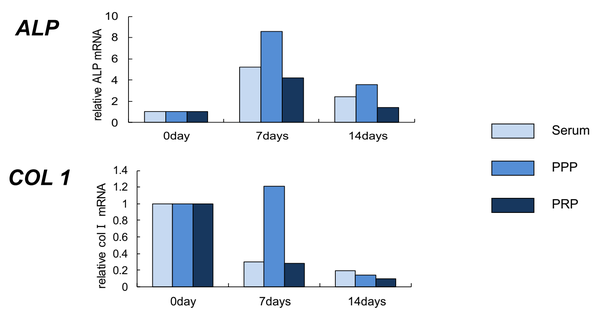
Effect of platelet-released growth factors on proliferation of hMSCs



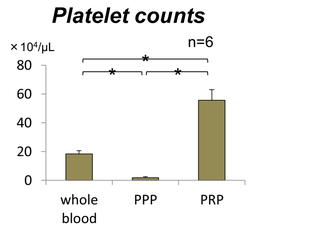
Effect of platelet-released growth factors on osteogenic differentiation of hMSCs

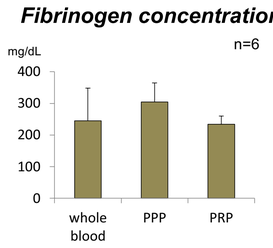


ALP, COL1 mRNA expression

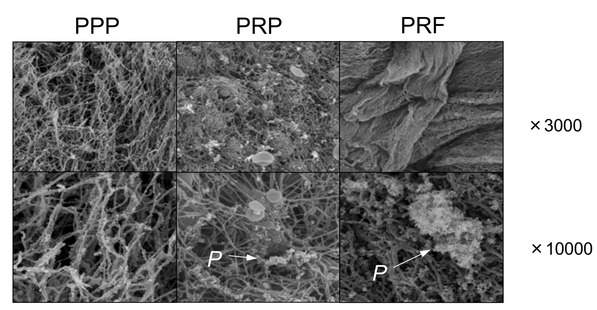


In vivo study





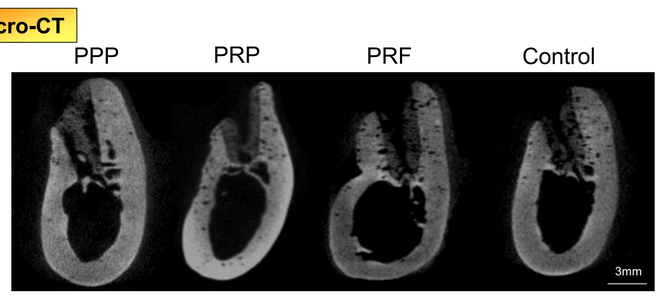
* Statistically significant differences with $p < 0.05$



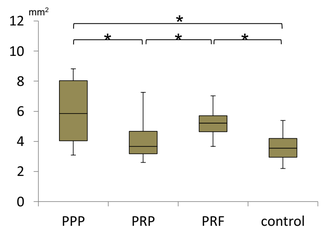
P; Platelet aggregates

In SEM analysis, the PRF group showed a more highly condensed fibrin fiber network that was regularly arranged when compared with the PPP and PRP groups. The fibrinogen concentration of PPP was higher than that of PRP.

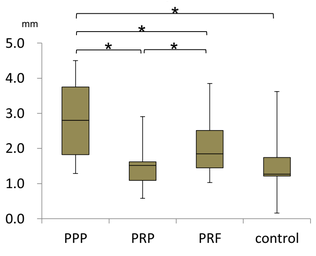
Micro-CT



Mean area of the newly formed bone at the crest of the alveolus

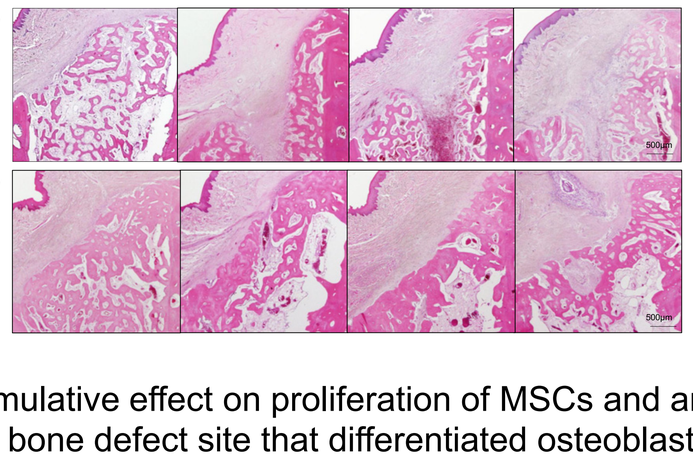


Mean horizontal bone width of a 1.5 mm lower part



* Statistically significant differences with $p < 0.05$

Histological findings



The amount of new bone formation in the extraction sockets was the highest in the PPP group at 4 weeks.

Eight weeks after surgery, PPP and PRF could sufficiently maintain the bone width and height for the preservation of the socket. However PRP could not maintain the bone width.
However, bone maturation in the PRF and the PRP groups was more progressed than that in the PPP and control groups.

The average number of days until epithelialization of extraction.(±SD)

PPP	PRP	PRF	Control
9.0±2.1	8.0±1.1	6.3±1.6	8.6±2.1

Soft-tissue healing was the fastest in the PRF group and slowest in the PPP group.

The alveolar crest appeared concave in four of six cases in the PRF group and all cases in the PRP and control groups, but it did not appear concave in any case in the PPP group.
The median new bone area and median horizontal bone width at 4 weeks after surgery were the highest in the PPP group.

【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.

The effects of growth factors

	PPP	PRF・PRP
Bone formation: Fewer osteogenic cells, The upper site of socket	promote ↑	inhibit ↓
Bone formation: Many osteogenic cells, The inner site of socket	inhibit ↓	promote ↑
Soft tissue regeneration	→	promote ↑

The effects of fibrin network

Fibrinogen concentration: PRP < PPP
Strength: PRP < PPP < PRF ?

↓

Space making: PRP < PPP < PRF ?

【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.