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A three-dimensional cell-loading system using autologous plasma loaded into a porous β-tricalcium-phosphate block promotes bone formation at extraskeletal sites in rats

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Abstract

The effects of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) on bone marrow stromal cells (MSCs) with respect to proliferation, osteogenic differentiation, and bone formation capability were investigated.

MSCs derived from rats were cultured in medium containing mixtures of PRP and PPP. Fibrinogen was eliminated prior to the experiment. The DNA content and alkaline phosphatase (ALP) activity were measured. PRP stimulated cell proliferation and inhibited osteoblastic differentiation. To examine the effects of fibrin in plasma, MSCs were cultured in PRP or PPP fibrin gels formed both on a cell culture insert installed in a culture well and on the bottom surface of the same culture well. The ALP activities of the MSCs in both of the gels were higher than those on the surface of the culture wells. The MSCs cultured on the PPP gel showed the highest ALP activity. The effects of PRP and PPP used as scaffolds for bone formation were also investigated. MSCs were suspended in PRP or PPP, introduced into porous β-tricalcium phosphate blocks, and then implanted into subcutaneous sites. Subsequently, bone formation was quantified. Further in vivo studies found that implants prepared using PPP had a greater osteoinductive capability than implants prepared with PRP.

Keywords: Platelet-rich plasma (PRP); Fibrin; Bone; Bone marrow stromal cells; Three-dimensional

1. Introduction

Autologous bone grafting is the most commonly used method for reconstructing a bone defect. However, potential complications include morbidity and donor site pain, as well as the risks of infection, nerve injury, and hemorrhage at the harvest site. Various kinds of osteoconductive artificial bone substitutes, such as hydroxyapatite-ceramics, β-tricalcium-phosphate (β-TCP), and other materials, have been developed in order to reduce the complications. However, the osteoinductive capacity of these alternatives has been found to be insufficient.

Bone marrow stromal cells (MSCs) are easily harvested by bone marrow aspiration and have the potential to expand and differentiate into an osteoblastic lineage [1–3]. Furthermore, the MSCs that are cultured in an appropriate scaffold with osteogenic supplements are able to induce bone tissue when implanted in vivo [4–6]. Based on the results of these basic studies, clinical bone reconstruction trials using this combination of materials have already been started [7].

In 1998, platelet-rich plasma (PRP) was reported by Marx et al. to promote new bone formation and to cause faster maturation

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when used with an autologous bone graft of the maxilla [8]. PRP is prepared from whole blood by centrifugation and contains concentrated platelets. Consequently, compared to whole blood, PRP has the potential to release more of the growth factors that are stored in platelets, such as TGF-β, PDGF, and VEGF [9,10]. Since PRP is an autograft, it has become frequently used in clinical bone repair surgery, especially in oral surgery. The use of PRP is based on the premise that platelet-derived growth factors and fibrinogen, which forms fibrin networks and is also present in PRP (though not in a concentrated manner), can both serve to regenerate bone tissue. However, there have been few basic studies dealing with PRP, and the mechanisms involved in bone regeneration remain unclear. Although some reports suggest that PRP has an effect on bone regeneration [8,11,12], other reports have concluded that PRP did not accelerate bone formation in vivo [14,15]. In vitro, PRP down-regulated alkaline phosphatase (ALP) activity of MSCs, and PDGF and TGF-β, which are abundant in PRP, have been reported to inhibit osteoblastic differentiation [13,16–19]. Thus, the effects on bone regeneration of the growth factors that are contained in PRP are controversial.

Fibrin and fibrin clots, which are thought to be beneficial to bone regeneration, play an important role in wound healing. By protecting the denuded wound tissues and providing scaffolds for cell migration during the tissue repair process, they provide a temporary shield. Furthermore, fibrin also serves as a reservoir for cytokines and growth factors [20–23]. Fibrin glue has also been reported to have a positive role in osteogenesis [24,25].

Three-dimensional cell growth is important for osteoblastic maturation and bone mineralization. Some trials have used gels made of various materials, such as fibrin, type 1 collagen, and sodium alginate, to act as a scaffold for cell growth. Their results indicated that the three-dimensional cell culture of osteoblasts promoted osteoblastic differentiation and mineralization [26–31].

In order to employ MSCs for tissue-engineered bone regeneration, we devised a cell-loading method using plasma to introduce MSCs into a porous artificial bone. Cells loaded with the plasma are held in a fibrin gel and develop three-dimensionally in the fibrin networks, whereas cells loaded into a culture medium can proliferate only along the surface of the pores (Fig. 1).

This study evaluated the effects of the plasma cell-loading method and compared the use of PRP with platelet-poor plasma (PPP) as the cell-loading scaffold.

2. Materials and methods

2.1. Preparation of PRP and PPP

All experiments were performed in accordance with the guidelines of the Ethics Committee of Tokyo Medical and Dental University (Tokyo, Japan). Under general anesthesia, the rats (F344, 9-week-old males) were given an intra-abdominal injection of trichloroacetaldehyde, their hearts were punctured, and blood (70 ml) was collected into sterile syringes containing 8 ml of the anticoagulant, citrate phosphate dextrose (CPD), (TERUMO, Japan). The whole blood was centrifuged at 312×*g* for 10 min, and the upper layers, including the buffy coat, were transferred to a new centrifuge tube to remove the lowest layer that contained most of the red blood cells and few platelets. After further centrifugation of the transferred layers at 1248×*g* for 10 min, 8 ml of PRP were collected from the bottom layer, which was rich in platelets. The upper layer, in which there were few platelets, was the platelet-poor plasma (PPP). The platelet concentrations in PRP, PPP, and whole blood were counted with a Coulter Counter (Beckman Coulter Co., Germany).

2.2. Preparation of plasma-derived growth factors

To 1 ml of blood products containing CPD (PRP, PPP, and whole blood), 167 μl of 2% calcium chloride were added to activate platelets and to convert fibrinogen to fibrin gel. The tubes were incubated for 4 h at room temperature to completely release the growth factors from the platelets, and then centrifuged at 1500×*g* for 10 min to precipitate fibrin gels. The supernatants, which included growth factors, were incubated at 4 °C for 1 day, filtered with a 0.2 μm pore filter (Acrodisc Syringe, Pall Co., NY,

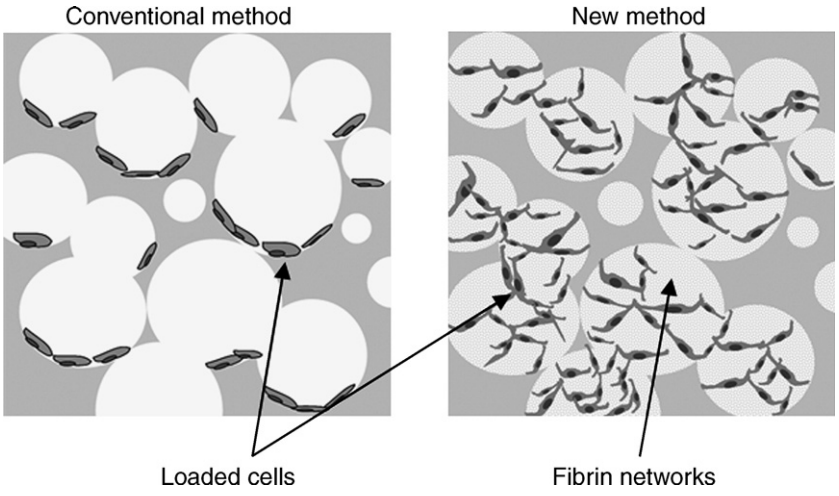

Fig. 1. Schematic drawings of cell-loaded β-TCP blocks. Cells loaded by the new method can develop three-dimensionally in fibrin networks, while cells loaded in the conventional way can proliferate only along the surface of the β-TCP.