Effect of Platelet-Rich Fibrin on Repair of Defect in the Articular Disc in Rabbit Temporomandibular Joint by Platelet-Rich Fibrin

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(Received: January 17th, 2011; Accepted: August 22nd, 2011)

Abstract: The objective of this study was to evaluate platelet-rich fibrin (PRF)'s effectiveness in repairing articular disc defect in the temporomandibular joint (TMJ) of rabbits. Eight rabbits were divided into four groups of two rabbits each, corresponding to groups A, B, C, and D. Both TMJs of all of the rabbits were used in the experiments: the right joints comprised the experimental groups, and the left ones, the control groups. The disc defect was circular and 2 mm in diameter. In the experimental groups, the PRF was compressed into the defect, whereas the control group defects were left untreated. A, B, C, and D groups were sacrificed at the 1st, 2nd, 4th and 6th weeks, respectively. The defects of each control group exhibited no specific changes. Contrastingly, in each experimental group, there was an increased number of chondroblasts at the margins of the defects, along with accelerated cell differentiation and a columnar cell arrangement observable at the time of cell differentiation. The experimental groups showed inflammatory cell infiltrations and fibrosis by the 1st week, maturation of chondrocytes by the 2nd week, and proliferation by the 4th week, after which the defects began to be filled with chondrocytes, a process that was complete after the 6th week. In the histological evaluation (H-E), the experimental groups showed significant increases of chondroblasts after the 2nd and 4th weeks, as well as regular columns of chondrocyte arrays observable during cell division. After 6 weeks, the defects were filled with chondrocytes.

Key words: temporomandibular joint, articular disc, platelet-rich fibrin (PRF)

1. Introduction

The temporomandibular joint (TMJ) is one of only two diarthrodial joints in the human body. It exhibits hinging, translational, and rotational movement. Histologically, the TMJ differs from other joints in its articulating surface, which, uniquely, is covered by fibrocartilage instead of hyaline cartilage. Fibrocartilage is dense, well-organized disc of collagen tissue having a very sparse population of cartilage cells.1 The disc plays an important role in maintaining the normal bony architecture of the TMJ and in preventing degenerative changes or ankylosis at the condyle of the mandible. If pathological changes persist, with resulting irreversible degeneration or perforation, surgical removal and rehabilitation of the disc might be necessary. The normal articular cartilage is avascular and alymphatic, except in the peripheral area, dictating that the disc depends on synovial fluid for its nutrient supply. If the TMJ is injured, there will be either a reduction or a total loss of that supply. Furthermore, if the stresses to which the disc is subjected are excessive, its surface will undergo degenerative changes including severe wear and deformation. There is only limited healing and little if any disc regeneration in the case of severe damage or pathological change.2

Platelet-rich fibrin (PRF) is a platelet concentrate collecting on a single fibrin membrane all of the constituents of a blood sample favorable to healing and immunity.3-5 Most recently, Choukroun et al. developed an autologous PRF product belonging to a new generation of platelet concentrates.6 They claimed that PRF is a slowly and naturally polymerizing fibrin matrix in which growth factors (PDGF-ß, TGFß-1, VEGF, and insulin-like growth factor-1), leukocytic cells, and their cytokines (interleukin[IL]-1ß, IL-6, IL-4, and tumor necrosis factor-α) are enmeshed.7,8 Some clinicians suggest that wound healing can be promoted through integration of growth factors originating from granules of platelets with autologous fibrin.9,11
Although platelets and leukocyte cytokines play an important role in the healing cascade, the fibrin matrix supporting them is certainly the operative element determining PRF’s real therapeutic potential.\textsuperscript{12,13} Fibrin plays a crucial role in the recruitment of neutrophils and monocytes, endothelial cells, and fibroblasts to the wound site. Also, the intrinsic characteristics of fibrin enable the cellular and humoral processes involved in epithelialization, granulation tissue formation, and angiogenesis.\textsuperscript{14-16}

A number of studies have been carried out to develop clinically useful procedures for restoration of destroyed articular cartilage;\textsuperscript{17-20} a satisfactory disc material, however, has proved elusive. The purpose of the present investigation was to evaluate the reparative effect of PRF on a defect in the rabbit TMJ articular disk.

2. Materials and Methods

2.1 Animals and Group Design

Eight New Zealand adult female rabbits older than 6 months and weighing between 2.5 and 3.5 kg were used in this study. The animals were housed individually in standard rabbit cages at the ambient temperature of 20°C and under a 12/12-hour light/dark cycle. The animals had free access to drinking water and standard laboratory feed pellets. This experiment (no. 2010-5) was approved by the Institutional Animal Care and Use Committee of Dong-A University Medical College, Busan, Korea. We divided the rabbits into four groups of two rabbits each, which were sacrificed at the 1st, 2nd, 4th, and 6th weeks, corresponding to groups A, B, C, and D, respectively. Both TMJs were used in the experiments, the right ones comprising the experiment groups and the left the control groups.

2.2 Surgical Method

The rabbits were anesthetized by intramuscular injection of Xylazine Hydrochloride (Rumpun®, Bayer, Korea), 20 mg/kg, and ketamine hydrochloride (Ketalar®, Yuhan, Korea), 4 mg/kg. The posterior area of the eye was shaved, and then disinfected with povidine-iodine. The exposed skin was incised, and the articular capsule was opened, exposing the articular disc. A circular, 2 mm-diameter defect was formed in the disc using a round bur (Fig 1A).

9 mm blood sample drew from rabbit’s ear vein and centrifuged. The PRF, the middle layer, was trimmed 3 mm in diameter. PRF was compressed into the defects in the experimental groups (Fig 1B), whereas the control groups received nothing. The articular capsule and skin were each closed using 4-0 vicryl and nylon sutures, respectively.

2.3 Implant Materials

A blood sample (9 mL) was obtained from the ear vein of each rabbit. Immediately afterwards, the dried monovettes (about 400 g; without anticoagulant) were centrifugated at 3000 rpm for 10 minutes in a laboratory centrifuge (Gyrozen, Gyrozen, Korea). The blood was then separated into three layers, among which the middle one, representing Choukroun PRF, was taken (Fig 2).

2.4 Histology

Each specimen was fixed in 10% formaldehyde solution and embedded in paraffin. Serial cross-sections (5 μm) were cut through the circular 2 mm diameter of the defect and stained with haematoxylin-eosin (H-E). The H-E stains revealed the cellular reaction indicating chondrocyte formation. The slides were photographed using a virtual slide system (Aperio Technologies ScanScope CS system; Aperio Technologies, USA).

2.5 Immunohistochemistry

Fibroblast growth factor-2 (FGF-2) (sc-79; Santa Cruz Biotechnology, USA) immunostaining was performed using