A Regular Protein Supply for Osteo Tissue Development a Platelet-Rich Blood Applicants

Abstract

The main purpose of this study is to investigate the effect of PRF scaffolds and autologous non-cultured bone marrow mononuclear cells on osteochondral regeneration in rabbit knees. Three different types of His-PRF scaffolds were generated from peripheral blood (Ch-PRF and L-PRF) and bone marrow in combination with uncultured bone marrow mononuclear cells (BMM-PRF). The histological properties of these scaffolds were evaluated using hematoxylin-eosin staining, picrosirius red staining, and immunohistochemical staining. An osteochondral defect 3 mm in diameter and 3 mm deep was developed in the trochlear groove of the femur of a rabbit. Different PRF scaffolds were then used to treat the defect. Regeneration of osteochondral tissue was assessed macroscopically (internal cartilage repair-related score, by X-ray) and microscopically (hematoxylin and eosin staining, safranin O staining, toluidine staining, histological Wakiya scale, immunohistochemistry) at 2 and 4 weeks. Post-assessed and 6-week post-assessment.

Keywords: Osteo tissue mononuclear cells • Platelet-rich fibrin • Tissue regeneration • Osteogenesisl

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Introduction

Regeneration of cartilage remains a challenge in tissue engineering as it lacks neural and vascular components and thus has limited recovery potential after injury. Regenerative medicine brings a broader perspective to the treatment of cartilage damage and includes three key integrated components: Although autologous chondrocytes are commonly used for articular cartilage regeneration, they have several drawbacks, including: B. Lack of cell source for large lesions and risk of dedifferentiation during in vitro culture [1]. However, scaling an MSC requires extensive facilities and technical expertise, which may not be available in many locations. Recently, autologous bone marrow mononuclear cells (BMMCs) have been used with various drugs as a promising cell therapy for various diseases [2]. Previous animal studies and clinical trials have demonstrated the efficacy and safety of BMMCs in treating cartilage lesions, are easier to isolate compared to MSCs, and do not need to be expanded in vitro before use [3].

These include a variety of growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), and transforming growth factors that help regenerate both soft and hard tissues. Due to its molecular structure and low thrombin concentration, it serves as a suitable matrix for migration of endothelial cells and fibroblasts. Moreover, its PRF and availability of autologous origin increase the utility of this material and minimize surgical time. Many authors point to his PRF applications in cartilage and tendon tissue engineering, which has led to an increase in in vitro, preclinical, and clinical studies [4]. Chondrogenesis is regulated by multiple cartilage-specific markers such as collagen, aggrecan (ACAN), SRY box transcription factor 9 (SOX9), and osteogenic markers such as alkaline phosphatase (ALPL) and bone gamma-carboxyglutamic acid protein (BGLAP). be characterized. Therefore, we conducted this study to investigate the effects of His PRF scaffolds and autologous uncultured BMMCs on osteochondral regeneration in rabbit knees [5].

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Methods

Animals

The use of rabbits in this study was approved by the Animal Ethics Committee of the University of Hue (certificate reference number: HU VN0010, November 10, 2021). They were randomly classified into four categories.

Nine rabbits were used to treat osteochondral defects with Shuclone platelet-rich fibrin (Ch-PRF) scaffolds and nine rabbits were used to treat osteochondral defects with leukocyte-rich fibrin (L-PRF) scaffolds. Did. Rabbits were used in experiments to treat osteochondral defects with scaffolds combined with uncultured bone marrow mononuclear cells and bone marrow-rich platelet fibrin (BMM-PRF) [6].

Preparation of Osteo tissue mononuclear cells

According to a modification of Choukroun's method, 9 ml of blood was collected from the rabbit central ear artery and immediately transferred to a 15 ml polypropylene centrifuge tube without anticoagulant. After centrifugation, the whole blood in each tube was separated into three layers.

Platelet-poor plasma (PPP), platelet-rich fibrin (PRF), and its base red blood cells. Plasma and red blood cells were discarded, the Ch-PRF scaffold was placed on a sterile perforated metal grid, and a light metal plate was placed on top of the gel to achieve a uniform thickness of 1 mm [7].

It was taken from behind the crest of the bone. Three milliliters of bone marrow was taken from each iliac crest and immediately mixed with the solution in the syringe (3 ml) by gentle swirling. A total of 12 mL of bone marrow solution from the two iliac crests was evenly transferred into two 15 mL conical tubes containing 3 mL of His Ficoll-Paque medium (density 1.077 g/mL) in each tube. These tubes were centrifuged at 2000 rpm for 20 minutes. After the initial centrifugation, the bone marrow sample was separated into four distinct layers.

The upper layer contains plasma, the buffy coat mainly contains bone marrow mononuclear cells, Ficoll-Paque fraction, and the lower layer mainly contains granulocytes and erythrocytes. The plasma and buffy coat layers were removed from the two tubes and placed in new 15 mL conical tubes. After a second centrifugation step, samples were separated from her BMMCs into plasma and pellet. The upper layer of plasma is called platelet-poor bone marrow plasma (PPP) and the lower layer is called platelet-rich bone marrow plasma (PRP). The platelet-poor plasma layer (PPP) of bone marrow was removed and the BMMC pellet was resuspended in PRP. Calcium chloride (10%) was then administered to a final plasma concentration of 0.1%. The BMM-PRF gel was placed on a sterile perforated metal mesh and a light metal plate was placed over the gel to ensure a uniform thickness of 1 mm [8].

Evaluation of the Osteo tissue regeneration

Histology of osteochondral defects 2, 4, and 6 weeks after surgery was analyzed by hematoxylin-eosin staining. After 2 weeks, defects in untreated rabbits were covered with fibrous connective tissue with relatively high concentrations of fibroblasts distributed throughout the matrix. In the experimental group, the implanted material was almost completely absorbed. There were no significant differences between these groups and the untreated group. Four weeks after surgery, he found that the density of fibrous connective tissue in the control group was reduced, with an underlying growth of adipose niches, chondrocytes, and cartilage matrix. No subchondral bone was present in this group. A similar phenomenon was observed in the experimental group. However, the formation of hyaline cartilage (chondrocytes and cartilage matrix) was more pronounced in his BMM-PFR group than in the untreated group. Moreover, in these groups, subchondral bone with discontinuous trabeculae occurred in the Ch-PRF and L-PRF groups and was more pronounced in the BMM-PRF group with continuous trabeculae [9]. Six weeks after his surgery, the remaining fibrous connective tissue was significantly thinner in the control group compared to the 4-week group. Cartilage and ossified bone appeared scattered and mottled, but somewhat prominent. Below, individual trabeculae of subchondral bone were seen. In the experimental group, the defect was more clearly covered with cartilage tissue than in untreated rabbits. In the Ch-PRF and L-PRF groups, immature chondrocytes were mainly located in the lower half of the

regenerated tissue. On the other hand, the regenerated tissue in the BMM-PRF group was almost similar to hyaline cartilage, and immature chondrocytes were distributed from the lower layer to the upper layer of the regenerated tissue [10].

Result

Heterogeneous fibrin network structure and cell populations. L-PRF and BMM-PRF had a homogeneous structure with a uniformly distributed fibrin network. Ch-PRF and L-PRF contained populations of CD45-positive leukocytes embedded in fibrin networks, whereas mononuclear cells within the BMM-PRF scaffolds responded to the pluripotent stem cell-specific antibody Oct-4 Did. It was positive. Rabbits implanted with autografts showed significantly improved articular cartilage and subchondral bone healing compared to the untreated group. A gradual recovery was observed after 2, 4, and 6 weeks of PRF scaffold treatment, which was particularly pronounced in the BMM-PRF group. Diploma: A combination of biomaterials containing autologous platelet-rich fibrin and uncultured bone marrow mononuclear cells promoted osteochondral regeneration in a rabbit model more than platelet-rich fibrin material alone. Our results suggest that autologous platelet-rich fibrin scaffolds combined with uncultured bone marrow mononuclear cells for healing osteochondral lesions may be a suitable adjunct to stem cell and biomaterial therapy.

Discussion

Biocompatibility is key to the successful application of tissue engineered tissue. Our study successfully demonstrated the biocompatibility and safety of autologous PRF scaffolds and uncultured BMMCs as transplanted biomaterials for in vivo cartilage repair. This is because these substances are autologous. No degenerative changes were observed in adjacent articular cartilage during the study period. Furthermore, neither inflammatory responses nor giant cells (common in inflammatory responses to foreign bodies) were observed in the implanted area of the experimental group. Two weeks after surgery, the graft was almost completely resorbed. Our results showed that rabbits treated with PRF scaffolds in combination with BMMCs had

a more pronounced effect on regeneration of osteochondral defects than controls and PRF alone. This can be demonstrated both macroscopically and microscopically.

PRF scaffolds have been shown to have a positive impact on osteochondral healing in terms of gross and histological findings. Many studies have been done using rabbits and other animal models. The therapeutic effect of PRF is primarily due to high concentrations of platelet-derived protein molecules that are primarily stored in and released from platelet α-granules. Among these, platelet-derived growth factors, including platelet-derived GF (PDGF), transforming GF-β1 (TGF-β1), and insulin-like GF (IGF-1), contribute significantly to chondrogenesis. By regulating cell proliferation, angiogenesis, inflammation, and extracellular matrix (ECM) deposition, they act as potent promoters of chondrogenesis and tendon. The dense network of polymerized fibrin formed by PRF increases the uptake of circulating cytokines and growth factors. These molecules are released slowly and have a long-lasting effect at the site of injury. Furthermore, this release is facilitated by the formation of new growth factors from membrane-bound PRF leukocytes. Among the different types of white blood cells, lymphocytes are more enriched than other white blood cells. Lymphocytes act as local regulators in the healing process, which may explain why her PRF membranes of lymphocytes continue to produce large amounts of growth factors over long periods of time.

Conclusions

In conclusion, autologous platelet-rich fibrin and uncultured bone marrow mononuclear cells work better together than platelet-rich fibrin material alone to promote osteo tissue regeneration in a rabbit model. In addition to stem cell and biomaterial therapy, using autologous platelet-rich fibrin scaffolds in combination with uncultured bone marrow mononuclear cells to cure osteo tissue lesions may be an effective strategy.

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