The Effect Of Fibrin Clot Derived From Bone Marrow On Human Meniscal Healing In An Organ Culture Model

Takeshi Shoji, Tomoyuki Nakasa, Naosuke Kamei, Takuma Yamasaki, Yuji Yasunaga, Mitsuo Ochi.

1Department of Orthopaedic Surgery, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan, 2Hiroshima prefectural rehabilitation center, Hiroshima, Japan.


Introduction: In an effort to preserve the important structures of the knee, many surgeons are considering primary repair of meniscal injury. However, the meniscus has a low healing potential, in some cases, primary repair has been unsuccessful.

An autologous fibrin clot is known to contain a variety of bioactive factors including growth factors and serve as a storage and release system for bioactive factors. In addition, the biological half-life of bioactive molecules may be prolonged after binding to the fibrin matrix. The plasticity of the material will allow adaptation to various tissue topographies during implantation, and the gel preparation techniques are suitable within an operation room. The therapeutic effects of autologous fibrin clot have been attributed both to their regenerative and trophic properties, the latter occurring through the production of bioactive products. An autologous fibrin clot derived from peripheral blood (pb-fibrin clot) was applied to meniscal injury and good results have been reported. Recently, fibrin clot derived from bone marrow (bm-fibrin clot) have been gradually applied clinically, and its effectiveness for tissue regeneration has been reported. However there has been no report to date, that compare pb-fibrin clot with bm-fibrin clot from the view point of the amount of growth factors and its effects of applying bm-fibrin clot on the success rate of meniscal repair. In this study we evaluated the amount of growth factors from human pb/bm-fibrin clot and prove the validity of bm-fibrin clot for meniscal healing.

Methods: Experimental Design

Three experiments were performed in this study. In the first experiment, for quantitative evaluation of the growth factors in pb-fibrin clot and bm-fibrin clot, enzyme-linked immunosorbent assay (ELISA) analysis was performed for detecting VEGF, EGF, IGF-1, FGF2, HGF, TGF-β, PDGF-AB and SDF-1 immediately after preparation of fibrin clot. Secondly, pb-fibrin clot and bm-fibrin clot was evaluated in vitro for the efficacy for the induction of meniscal cell proliferation using human meniscus cells which was resected intra-operatively such as meniscectomy. Human meniscal cells were seeded on plates and maintained in Dulbecco’s modified Eagle’s medium (DMEM: Ham’s F12 (1:1)) containing 10% fetal bovine serum and 1% antibiotic. To evaluate meniscal cell proliferation, pb-fibrin clot and bm-fibrin (100 μl/ml) clot was added, and immunohistochemistry of Collagen type2(Col2) and real time PCR of Col2A1 was performed at 4days after addition of fibrin clot. Evaluations were performed among the control group which was nothing transferred, pb-fibrin clot group and bm-fibrin clot group. Third, the 1mm hole was created in the avascular portion of the meniscus and putted fibrin clot in the hole, and was cultured in the same medium for organ culture. Histological evaluation, immunohistochemistry and real time PCR of Col2A1 and terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling
Preparation of pb-fibrin clot and bm-fibrin clot constructs
A human blood who underwent surgery was obtained for preparation of fibrin clot. The patients included a total of 20 patients who underwent surgery such as total hip arthroplasty or osteotomy, including 2 men and 18 women with a mean age of 53.6 years (range, 36-65 years). The diagnoses were osteoarthritis of the hip and none of the patients were treated with anticoagulant drugs. A total of 20 ml peripheral blood bone was obtained from the median vein of the right upper extremities and same volume of bone marrow aspirate was obtained from the anterior iliac crest during an operation. Peripheral blood clot or bone marrow placed in sterile grass and stirred with a glass rod. Then almost 2-3ml of clot material was precipitated in about 3-5 minutes.

Fabrication of meniscal cells
Meniscus were harvested from human knee joints intra-operatively. After cutting the meniscus into pieces, the tissue was digested with trypsin for 30 minutes followed by 0.25% collagenase for 4 hours at 37°C. After passing through a cell strainer, the cells were suspended in culture medium.

Results: Eliza analysis revealed that the amount of growth factors such as VEGF, HGF, FGF and IGF-1 in bm-fibrin clot group was higher than those in fibrin clot group with statiscal differences. However, there were no significant differences in EGF, TGF-β and PDGF. (Figure.1) In the second experiment, real time PCR analysis revealed that the expression levels of Col2a1 were significantly higher in bm-fibrin clot group, and immunohistochemistry also confirmed that Col2 was highly upregulated in bm-fibrin clot group compared with other two groups. (Figure. 2) In the third experiment, histological analysis confirmed that the transected area was covered with healing tissue in bm-fibrin clot group, but remained sparse of any tissues in pb-fibrin clot group at 3 weeks. Real time PCR analysis revealed that Col2a1 was highly expressed compared to other two groups.

Discussion: The meniscal healing can be affected by many factors such as the surrounding tissues, blood supply, nutrient delivery, biomechanical forces, and the supply of several growth factors. Several approach has been taken for tissue regeneration, indeed, the combination of cells and scaffolds could represent the optimal solution in the management of many orthopedic fields, potentially reducing complications and healthcare costs. The need for cell attachment, proliferation, and differentiation in tissue engineering remains crucial and adequate scaffolds, preferably bio-absorbable are required. An autologous fibrin clot is known to contain a variety of bioactive factors and offers many advantages in terms of availability, biocompatibility and biodegradation. The fibrin molecules of the fibrin clot can serve as a storage and release system for bioactive factors and in addition, fibrin clot is a suitable carrier for MSCs, showing clot-entrapped MSCs to be housed in a fibrin-microfiber porous-like structure. Therefore it can be thought as good cell source and scaffold for regenerative applications. In this study, it was revealed that the amount of growth factors in bm-fibrin clot are higher than that of pb-fibrin clot in VEGF, HGF, bFGF, IGF-1 and SDF-1 with statistical differences. These growth factors were known to be an important and essential for angiogenesis bias stimulating endothelial progenitor cell, proliferation and stimulating of fibroblasts and myoblasts, which plays a critical role in the meniscal regeneration. Besides, we confirmed that bm-fibrin clot has much potential for the proliferation of meniscal cells compared with pb-fibrin clot, and addition of bm-fibrin clot was more effective in promoting the healing of partially injured meniscus through enhancement of several growth factors.
In conclusion, the positive outcome confirms the efficacy of bm-fibrin clot for meniscal healing. Although further comprehensive studies are needed to determine its suitability as a therapeutic agent, the current study showed the possibility of a strategy for regenerative medicine by using bm-fibrin clot. **Significance:** This study suggests that bm-fibrin clot is effective for meniscal healing clinically.
Collagen type II

A) Immunohistochemistry

B) Real time PCR (*: P<0.05)

Figure 2

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