Autologous Fibrin-Base Scaffold for Chondrocytes and Bone Marrow Mesenchymal Stem Cells Implantation: The Development and Comparison to Conventional Fibrin Glue

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Objective: *The authors developed the autologous fibrin-base scaffold for chondrocytes and bone marrow mesenchymal stem cells (BM-MSCs) implantation and evaluated cells viability in autologous fibrin-base scaffold comparing to commercial fibrin glue.*

Material and Method: *The chondrocytes and BM-MSCs were seeded into autologous fibrin-base scaffold and commercial fibrin glue. The cell viability and proliferation were evaluated at 1 and 7 days. The histology were evaluated with hematoxylin-eosin (H&E) staining and cartilaginous matrices formation with Alcian blue, Saffanin-0, Toluidine blue, and Collagen type II staining at 6 weeks. The fixation of the scaffolds was observed.*

Results: *The chondrocytes and BM-MSCs could not survive in commercial fibrin glue. The chondrocytes and BM-MSCs in autologous fibrin-base scaffold could proliferate and synthesize the cartilaginous matrices on Alcian blue, Saffanin-0, Toluidine blue, and Collagen type II staining at 6 weeks. The fixation strength is excellent.*

Conclusion: *The developed autologous fibrin-base scaffold can be used as the scaffold for chondrocytes and BM-MSCs implantation with potential to implant chondrocytes and BM-MSCs arthroscopically.*

Keywords: *Knee injury, Autologous chondrocytes implantation, Bone marrow mesenchymal stem cells (BM-MSCs), Fibrin-base scaffold*

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The articular cartilage injury is a common injury. The healing capacity of articular cartilage is limited because of the absence of blood supply, low mitotic activity, and immobility of articular chondrocytes[1,2]. The conventional procedures (abrasive chondroplasty, subchondral drilling, and microfracture) had been reported with replacement with fibrocartilage and eventually following with premature degeneration[3]. The autologous chondrocyte implantation (ACI) had been developed using the expanded autologous chondrocytes to implant into the cartilage defects[4-6]. The patients required the first arthroscopic harvest of the cartilage, and second implantation of scaffold seeded with chondrocytes. The autologous chondrocytes implantation (ACI) using the scaffold is the treatment for large cartilage defects. The fixation is suturing the periosteal flap. The proper scaffold and fixation are being investigated. Bone marrow mesenchymal stem cells (BM-MSCs) are multipotent, being capable of differentiation to chondrocytes[7,8]. The BM-MSCs have the high potential to be the cell source for cartilage repair

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procedure[9-12]. Fibrin consists of the blood proteins fibrinogen and thrombin which participate in blood clotting. Fibrin glue or fibrin sealant is also referred to as a fibrin based scaffold and used to control surgical bleeding. The autologous fibrin-base scaffold such as fibrin glue could be the alternative as a scaffold and provide the good fixation. However, the biocompatibility of scaffold is crucial. The authors developed the autologous fibrin-base scaffold for chondrocytes and bone marrow mesenchymal stem cells (BM-MSCs) implantation, evaluated survival and proliferation of chondrocytes and BM-MSCs in autologous fibrin-base scaffold comparing to commercial fibrin glue and fixation strength.

Material and Method

Human mesenchymal cells (MSCs), chondrocytes, and fibrin-base scaffold were isolated from aspiration of bone marrow, arthroscopic harvesting of the cartilage, and blood collection by venipuncture respectively of the same subjects receiving operation at the HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakhrinwirot University. The eight subjects were included. This study received an ethical approval from the Institutional Review Board of the Srinakhrinwirot University. All subjects gave written informed consent prior to the sample collection.

Chondrocytes isolation

Knee arthroscopy was performed. The slivers of cartilage (300 mg) were obtained from the minor load-bearing area on the upper lateral or medial femoral condyle of the knee. The cartilage were minced and transferred to the laboratory in DMEM (Gibco BRL) at ambient temperature. The chondrocytes isolation was initiated not later than 6 hours after the harvesting. The cartilage was washed twice in Ham's F-12 medium (Gibco BRL, Paisley, Scotland) supplemented with gentamicin sulfate (50 µm/mL), amphotericin B (2 µm/mL), and L-ascorbic acid (50 µm/mL). The minced cartilage was digested 16-20 hours with clostridial collagenase (0.8 µm/mL, catalog No. C-9407, >1,200 IU/mg; Sigma, Freehold, New Jersey) and deoxyribonuclease (0.1 µm/mL, catalog No. D-5025; Sigma). The isolated cells were resuspended in culture medium containing DMEM/F12 1:1 (Gibco BRL) with 10% bovine serum (FBS) (Gibco BRL, NY, USA) and gentamicin sulfate (50 µm/mL), amphotericin B (2 µm/mL), L-ascorbic acid (50 µm/mL), and L-glutamine (Gibco BRL). The chondrocytes were incubated in 5% CO_2, in air at 37°C. Upon 90% confluent, they were trypsinized by 0.05% trypsin (Gibco BRL) and resuspended for adequate number of cells[5,6]. The quality-control procedures consist of sterility testing and photographic recording of cell morphology[13,14].

BM-MSCs isolation

Heparinized bone marrow samples (80 ml) were obtained by aspiration from anterior iliac crest under local anesthesia and transferred to the laboratory at ambient temperature. Bone marrow mononuclear cells were separated by density gradient centrifugation with 1.073 g/ml Percoll solution (Sigma, MO, USA). Briefly, 10 ml of heparinized bone marrow cells were mixed in an equal volume of Dulbecco's Modified Eagle's Medium (DMEM) (BioWhittaker, USA) and centrifuged at 900 g for 10 min at room temperature. The washed cells were re-suspended in DMEM, and 5 ml aliquot was layered over 1.073 g/ml Percoll solution and centrifuged at 1,000 g for 30 min at room temperature. The interface mononuclear cells were collected and washed twice with DMEM. Total cell count and viability were evaluated by 0.2% Trypan blue exclusion. The BM-MSCs were cultured in DMEM with 10% fetal bovine serum (FBS) (Gibco BRL, NY, USA) and gentamicin sulfate (50 µm/mL), amphotericin B (2 µm/mL) at 37°C, 5% Co_2. On day 3 of cultivation, non-adherent cells were discarded and this process was repeated every 4 days. Upon 90% confluent, BM-MSCs were trypsinized by 0.05% trypsin (Gibco BRL) and passaged for expansion[7,8]. The quality-control procedures consist of sterility testing and photographic recording of cell morphology.

Fibrin-base scaffold preparation

Autologous fibrinogen was precipitated from blood collected by venipuncture into 0.2 M sodium citrate. The 100 ml of autologous blood was centrifuged at 4,500 g for 15 min (4°C), placed in a freezer at -30°C for 16 hours, and centrifuged to obtain fresh plasma and to sediment the precipitate at 6,000 g for 10 min (4°C) for the fibrinogen-rich concentrate.

Chondrocytes and BM-MSCs in the scaffold

The $5x10^4$ cells each of chondrocytes and BM-MSCs were mixed into commercial fibrinogen (Tisseel; Immuno AG, Vienna, Austria) and autologous fibrin-base scaffold in the 6-well plate. The thrombin (Tisseel; Immuno AG, Vienna, Austria) were added to form the fibrin glue in both groups. The fixations of fibrin glue were observed. The cell appearances (viability and proliferation) were evaluated from light microscope

at 1 and 7 days. The histology of commercial fibrinogen and autologous fibrin base fibrin-base scaffold seeded with chondrocytes and BM-MSCs were evaluated with hematoxylin-eosin (H&E) staining and cartilaginous matrices formation with Alcian blue, Saffanin-0, Toluidine blue, and Collagen type II staining at 6 weeks.

Results

The chondrocytes and BM-MSCs could not survive in commercial fibrinogen. The cell death and apoptosis were observed from seeding the chondrocytes (Fig. 1) and BM-MSCs (Fig. 2) in commercial fibrinogen at 7 days. However, autologous fibrin-base scaffold seeded with chondrocytes and BM-MSCs showed cell viability with proliferation at 7 days (Fig. 3). The autologous fibrinogen-rich concentrate can form well-forming cartilaginous graft with excellent fixation (Fig. 4). The histologic evaluations at 6 weeks of commercial fibrin glue seeded with BM-MSCs and chondrocytes in H&E staining showed no cell viable; comparing to autologous fibrin-base scaffold with BM-MSCs and chondrocytes showed viability of BM-MSCs and chondrocytes with cell proliferation (Fig. 5). The histologic evaluations at 6 weeks of fibrin-base scaffold seeded with chondrocytes for cartilaginous showed cartilaginous matrices formation in Alcian blue, Saffanin-0, Toluidine blue, and Collagen type II staining.

Discussion

Several natural biomaterials, such as

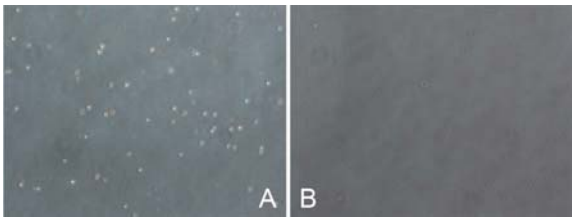

Fig. 1 The chondrocytes in commercial fibrin glue at 1 day (A), and 7 days (B) showed cells death.

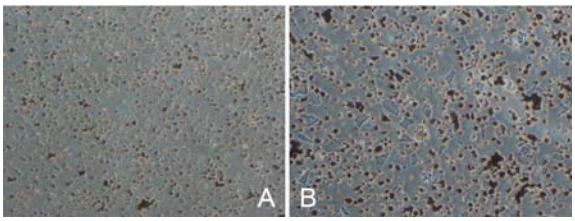

Fig. 2 The BM-MSCs in commercial fibrin glue at 1 day (A), and 7 days (B) showed cells apoptosis.

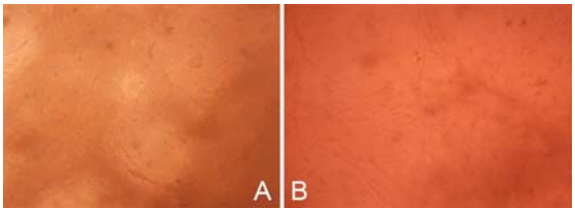

Fig. 3 The chondrocytes in autologous fibrin-base scaffold (A), BM-MSCs in autologous fibrin-base scaffold (B) at 7 days showed viability with cells proliferation.

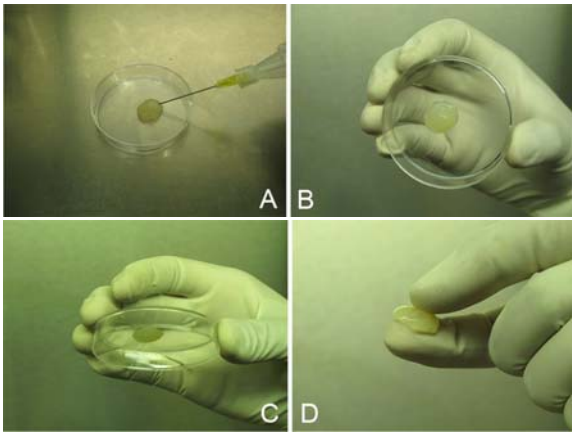

Fig. 4 The fibrin-base scaffold has the excellent fixation (A-C) with well-forming graft (D).

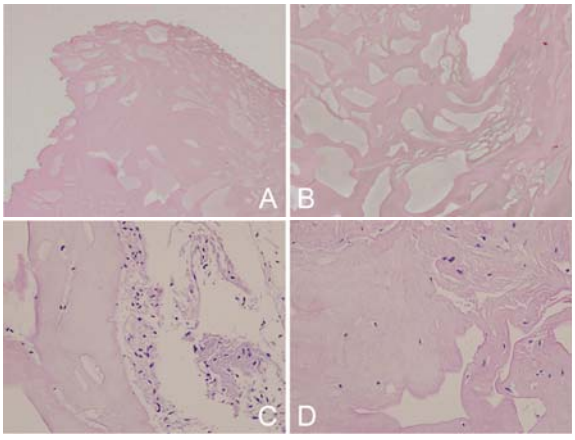

Fig. 5 The histology at 6 weeks of commercial fibrin glue seeded with BM-MSCs (A) and chondrocytes (B) in H&E staining showed no cell comparing to autologous fibrin-base scaffold showed viability of BM-MSCs (C) and chondrocytes (D).

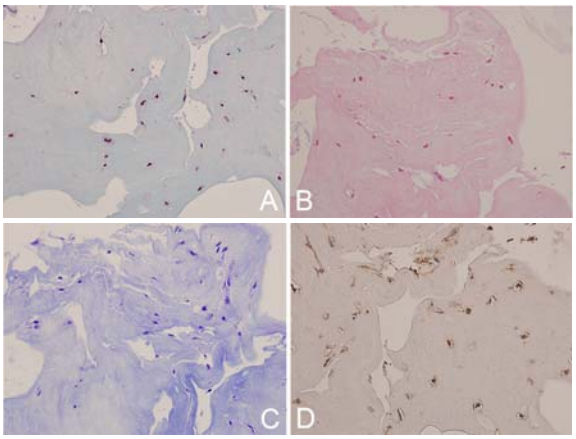

Fig. 6 The histology at 6 weeks of fibrin-base scaffold seeded with chondrocytes showed matrices formation in Alcian blue (A), Saffanin-0 (B), Toluidine blue (C), and Collagen type II (D) staining.

collagen[13-16], hyaluronan[17], fibrin glue[18,19], alginate[20], agarose[21], chitosan[22], as well as synthetic biomaterials such as polylactic acid[23] have been developed for the restoration of damaged cartilage. The scaffold should be biocompatible, mechanically stable, and biodegradable. The scaffold should allow successful infiltration and attachment with appropriate bioactive molecules in order to promote cellular differentiation and maturation. The synthetic and xenograft materials can cause the inflammation, immune response , toxicity, and cellular induction. They also need the techniques for graft fixation. A fibrin is a network of protein that holds together and supports a variety of living tissues naturally by the body after injury. The autologous fibrin-base scaffold has no undesired immunogenic reactions in addition to be reproducible. The study showed the simple method for preparing autologous fibrinogen for chondrocytes and BM-MSCs implantation. The immunogenic reactions and preparation could be the factors for chondrocytes and BM-MSCs viability in commercial fibrin glue. The previous studies showed the chondrocytes could survive in the fibrin glue gel. On the other hand, the biomechanical properties remained steady[18,19]. Our study showed the developed autologous fibrin-base scaffold could provide the good cell viability and proliferation for chondrocytes and BM-MSCs. The production of cartilaginous matrices was excellent. The autologous fibrin-base scaffold has the biodegradable potential with stable temporary fixation to allow cellular differentiation and maturation. The periosteal suturing graft fixation could have periosteal hyperthophy which is the undesired complication[24]. The autologous fibrin-base scaffold needs no periosteal fixation, which allow to do BM-MSCs implantation with simple, minimal invasive arthroscopically technique.

Conclusion

The authors developed the proper autologous fibrin-base scaffold that can be used with chondrocytes and BM-MSCs implantation. Both cells can survive and proliferate. The excellent fixation strength has the potential to do autologous chondrocytes and BM-MSCs implantation arthroscopically.

What is already known on this topic?

The conventional fibrin glue is used to stop the bleeding by adhering to the bleeding site.

What this study adds?

The chondrocytes and BM-MSCs can proliferate and synthesize the cartilaginous matrices in developed autologous fibrin-base scaffold. The developed autologous fibrin-base scaffold can be used as the scaffold for chondrocytes and BM-MSCs implantation with potential to implant chondrocytes and BM-MSCs arthroscopically.

Acknowledgements

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Potential conflicts of interest

None.

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การพัฒนาและศึกษาเปรียบเทียบระหว่าง Autologous Fibrin-Base Scaffold กับ Commercial Fibrin Glue ในการปลูกถ่ายเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูก

ชาญณรงค์ เกษมกิจวัฒนา, วิศิษฎ์ รังษิณาภรณ์, ธนา ศิริพิสิฐศักดิ์, พิงควรรส คงมาลัย, รณชิต บุญประเสริฐ, ชัชวาลย์ เจริญธรรมรักษา, สุรเดช หงส์อิง, อดิศักดิ์ วงษ์ขจรศิลป์, สรนาท เมืองสมบูรณ์, กานดา ชัยภิญโญ, โกสุม จันทร์ศิริ

วัตถุประสงค์: เพื่อพัฒนา autologous fibrin-base scaffold ใช้ในการปลูกถ่ายเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูก และศึกษาเปรียบเทียบการเพาะเลี้ยงกระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูกใน autologous fibrin-base scaffold และ commercial fibrin glue

วัสดุและวิธีการ: เพาะเลี้ยงเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูกใน autologous fibrin-base scaffold ที่สกัดได้จากผู้ป่วย และ commercial fibrin glue วัดผลการอยู่รอดและการเจริญเติบโตของเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูก และศึกษาผลชิ้นเนื้อการแบ่งตัวจากการเพาะเลี้ยงเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูกใน autologous fibrin-base scaffold และ commercial fibrin glue และการสร้างโปรตีนกระดูกอ่อนด้วยการย้อม Alcian blue, Saffanin-0, Toluidine blue, Collagen type II

ผลการศึกษา: พบว่าเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูกไม่สามารถมีชีวิตและเจริญเติบโตได้ใน commercial fibrin glue ส่วน autologous fibrin-base scaffold สามารถเพาะเลี้ยงเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูก มีการพัฒนาสร้างโปรตีนกระดูกอ่อน และยังมีคุณสมบัติในการเกาะยึดติดกับรอยโรคได้เป็นอย่างดี

สรุป: สามารถพัฒนา autologous fibrin-base scaffold ซึ่งนำไปใช้ในการปลูกถ่ายเซลล์กระดูกอ่อนและเซลล์ต้นกำเนิดจากไขกระดูกด้วยวิธีส่องกล้องได้