

Combination of Intra-Articular Autologous Activated Peripheral Blood Stem Cells with Growth Factor Addition/Preservation and Hyaluronic Acid in Conjunction with Arthroscopic Microdrilling Mesenchymal Cell Stimulation Improves Quality of Life and Regenerates Articular Cartilage in Early Osteoarthritic Knee Disease

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Background: Trauma or osteoarthritis (OA) create articular cartilage defects that cannot efficiently heal, thus leading to significant long-term disability. Failed conservative treatment in cartilage diseases is a known condition that necessitates repair attempts but current methods are inadequate. Recent studies in OA animal models and humans, showed articular cartilage regeneration following combinations of drilling, adult stem cells, and intra-articular hyaluronic acid.

Objective: In the present series, the authors evaluated the combination of repeated intra-articular (IA) autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with hyaluronic acid (HA) in conjunction with arthroscopic microdrilling mesenchymal cell stimulation (MCS) in early osteoarthritic knee disease that failed conservative treatment.

Material and Method: Four women and one man (median age 56, range 52-59 years) that failed conservative treatment were enrolled. Arthroscopic MCS was performed once in all patients with subsequent IA injection of AAPBSC with GFAP along with IA-HA intra-operatively, repeated at days 7 and 14. The patients were evaluated by WOMAC and KOOS scores at baseline, one, and six months. Cancellous bone biopsies were performed to investigate cell attachment, proliferation, and differentiation by electron microscopy and histological staining.

Results: All patients improved significantly in WOMAC and KOOS scores at one and six months compared to baseline. No adverse effects were seen during the AAPBSC harvesting, arthroscopy and/or IA injections. One month post-surgery, all pain medications could be withdrawn. Electron microscopy scanning revealed cell attachment and proliferation while histological analysis demonstrated that the cell layer on the cancellous scaffold showed increased proteoglycan and glycosaminoglycan content indicating hyaline cartilage presence.

Conclusion: The combination of intra-articular (IA) autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with hyaluronic acid (HA) in conjunction with arthroscopic microdrilling mesenchymal cell stimulation (MCS) resulted in Quality of Life improvements measured by WOMAC and KOOS scores and succeeded in regenerating articular cartilage in early osteoarthritic knee disease that failed conservative treatment. Further controlled studies are warranted to confirm the above results in larger groups.

Keywords: Peripheral blood, Stem cells, Hyaluronic acid, Osteoarthritis, Knee arthroscopy, Granulocyte colony stimulating factor, hG-CSF, Leukapheresis, WOMAC, KOOS

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Articular hyaline cartilage is a specialized, low-friction, and wear-resistant surface tissue in weight bearing, diarthrodial joints with its main function to

absorb, cushion, and protect the underlying bone from forces generated while a joint is being used. Its avascular, alymphatic nature complicates and often gravely impedes its capacity for regeneration and self-healing. Trauma or osteoarthritis (OA) creates full-thickness chondral defects that cannot efficiently heal, thus leading to significant long-term disability. Failed conservative treatment in cartilage diseases is

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a known condition that necessitates repair attempts. At that point, current choices of treatment like arthroscopic microfracture (MCS), autologous chondrocyte implantation (ACI), high tibial osteotomy, or unilateral knee arthroplasty are generally limited in their ability to regenerate functional cartilage⁽¹⁾. Research in recent years has focused on tissue engineering solutions in which the regeneration of cartilage is pursued through combinations of cells (e.g., chondrocytes or stem cells), alone or paired with scaffolds (e.g., hydrogels, sponges, and meshes) in conjunction with stimulatory growth factors and bioreactors^(2,3). In young patients or in those with early OA, attempts can be made to save articular cartilage until a more appropriate time for surgery arises⁽²⁾. Autologous chondrocyte implantation may be considered but has disadvantages such as poor cartilage integration between the recipient site and chondral graft⁽⁴⁾ as well as hypertrophy of the chondral graft resulting in irregular joint surface⁽⁵⁾, precipitating double surgical procedures and increased costs. Arthroscopic debridement/micro-drilling alone is not recommended for OA since there appear to be no benefits compared to a sham operation⁽⁶⁾. Patients treated with intra-articular hyaluronic acid injections (IA-HA) show clear symptomatic improvement and the intervention appears cost effective in up to two years follow-ups⁽⁷⁾ but whether cartilage regeneration actually occurs is open to debate⁽⁸⁾. Neither autologous chondrocyte implantation nor microdrilling alone seems to result to cartilage resurfacing histologically resembling normal articular cartilage⁽⁹⁾. Recent studies have studied the regenerative capacity of bone marrow stem cells in an OA animal model⁽¹⁰⁾ and peripheral blood stem cells in humans⁽¹¹⁾, in contributing to articular cartilage regeneration following combinations of drilling and IA-HA while others employed intra-articular autologous adipose tissue derived stem cells and combinations of growth factors⁽¹²⁾ with encouraging results. The resulting cartilage texture is still debatable and there is paucity in the basic understanding of cartilage regeneration such as gene expression and electron microscopic visualization. Moreover, the use of bone marrow stem cells is limited by the fact that collection is invasive, painful, the cells require processing before use, and collected numbers are often inadequate, not allowing for repeat implantation. Apart from the above, autologous adipose tissue derived stem cells face even more obstacles as their safety has not yet been established but are currently used in procedures such volume expansion in plastic surgery⁽¹³⁾.

The purpose the present study was to elucidate the effects of a novel adult stem cell preparation in articular cartilage regeneration and quality of life as measured by WOMAC and KOO scores. Our adult stem cell preparation consisted of human granulocyte colony stimulating factor (hG-CSF) activated autologous peripheral blood stem cells (AAPBSC) obtained through a minimally invasive procedure of leukapheresis. hG-CSF is a 20-kDa glycoprotein and is well known to stimulate the proliferation, survival and maturation of cells committed to the neutrophil precursors and matures neutrophils through binding to specific G-CSF receptors⁽¹⁴⁾. Intra-articular hG-CSF may further enhance cartilage regeneration as it has recently been proven to contribute to angiogenesis in an appropriate environment and regulate the proliferation, migration, or differentiation of the adult mesenchymal stem cells⁽¹⁵⁾ locally. Intra-articular hG-CSF was therefore, mixed with autologous platelet rich plasma (PRP) proven to preserve crucial growth factors such as transforming growth factor (TGF) beta, insulin-like growth factor (IGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), all known to promote stimulation of bones, blood vessel and chondrocyte formation⁽¹⁶⁻¹⁸⁾. This innovative mixture was ultimately combined with intra-articular hyaluronic acid (IA-HA), which along with the blood clot from the microdrilling served as a natural scaffold to effectively hold the preparation in place.

This case series demonstrates successful clinical and laboratory results using the combination of intra-articular (IA) autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with hyaluronic acid (IA-HA) in conjunction with arthroscopic microdrilling mesenchymal cell stimulation (MCS) in regenerating articular cartilage in early osteoarthritic knee disease that failed conservative treatment.

Material and Method

Patients

Between March 1 and May 31, 2011, five patients were enrolled in the present study. The present study protocol was approved by the Ethics Committee of the Police General Hospital and the Thai Medical Council. All patients signed informed consent forms after having received adequate information on the PBSC collection and discussed the procedure with the treating surgeon.

The diagnosis of OA and cartilage disease was made using clinical, radiological, and arthroscopic

evaluation. The diagnosis of chondral injury was made after clinical and radiological evaluation. Chondral lesions were graded according to the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package⁽¹⁹⁾. The inclusion criteria were OA of the knee classified as Kelleghan Lawrence stages 1-3, ICRS grade III and IV lesions, failed conservative treatment for more than six months, no more than 3-degrees varus or valgus deformity, VAS >40. Exclusion criteria were, age older than 60 years, secondary osteoarthritis, plica, intra-articular loose bodies, inflammatory joint disease, intra-articular steroid injection within the last six months, intra-articular hyaluronic acid within the last three months, prior glucosamine sulphate, varus deformity more than 3-degrees, severe osteoarthritis (Kelleghan Lawrence stage more than 4) known malignancy, allergy to hyaluronic acid, allergy or know prior reaction to hG-CSF, severe or uncontrolled diabetes and/or proliferative diabetic retinopathy, obesity with BMI greater than 30, and prior stem cell treatment. All patients have been followed-up for a median of six months (range 6-12 months).

Procedure outline

The protocol comprised three steps. First, hG-CSF AAPBSC was harvested and GFAP concentrate was prepared from autologous platelet rich plasma (PRP) and was mixed with hG-CSF. Then, on the following day, modified arthroscopic MCS technique with intra-operative injection of the above fresh solution took place. Finally, serial IA injections of the previous frozen-thawed AAPBSC preparation and fresh prepared GFAP on post op day 7 and 14.

Activated autologous peripheral blood stem cell (AAPBSC) harvesting and cryopreservation

Autologous PBSC were harvested by leukapheresis in a Cobe Spectra apheresis machine (Caridian BCT, Denver, CO) following 5-day stimulation with subcutaneous injection of hG-CSF at a dose of 5 µg/kg BW/day. Following the harvest, PBSC were tested for sterility (bacterial, viral and fungi) and after a fresh aliquot of 3 ml was reserved, part of the remaining portion was cryogenically frozen in 10% dimethyl sulphoxide and preserved in two cryovials of 4.5 ml to be thawed at the desired time of the future intra-articular injections. Pre- and post-thaw measurements of viability and total nucleated cells (TNC) and CD34+ indicating hematopoietic stem cell markers and CD105 indicating mesenchymal stem cell

markers were performed via flow cytometry (Beckman Coulter, Fullerton, CA). Prior to each weekly intra-articular injection, each aliquot of PBSC was checked for appropriate cell counts and viability as per above.

Arthroscopic microdrilling mesenchymal cell stimulation (MCS) procedure

A single surgeon (TT) performed all arthroscopic procedures. The patients were scheduled for arthroscopic debridement, identification of the pathologic lesion(s), removal of calcified layers with preservation of subchondral bone, creation of a stable rim of good cartilage, and multiple drillings of 2.00 mm at a depth of 4 to 6 mm. Then, immediately after the above procedure, intra-operatively and under aseptic conditions, 3 ml of hG-CSF AAPBSC was injected in the articular space, followed by 2 ml of GFAP concentrate prepared from autologous platelet rich plasma (PRP) mixed with hG-CSF at a dose of 5 µg/kg BW. Two ml of HA was finally injected (Ostenil, MW = 1.2 Mdal) IA. No drain was inserted nor was any anesthetic infiltration used. During above initial arthroscopic procedure, core cancellous bone at a diameter of about 2x4 mm was biopsied for gene expression, electron microscope, and histological staining studies.

On post-operative day 7, and day 14, the second and third IA injections were given, after thawing 4.5 ml of cryopreserved hG-CSF AAPBSC followed by 2 ml of GFAP concentrate, fresh prepared from autologous platelet rich plasma (PRP) on that same day mixed with hG-CSF at a dose of 5 µg/kg BW. Two ml of HA was finally injected (Ostenil, MW = 1.2 Mdal) IA at each occasion.

Patient demographics, WOMAC (WOMAC 3.1®; www.womac.org) and Knee injury and Osteoarthritis Outcome Score (KOO; <http://www.koos.nu/>) were recorded preoperatively and at the one and six month follow-up. The patients were allowed to non-weight bearing ambulation with axillary crutch and were discharged on the same day. All patients were monitored and telephoned on the day after their treatments. A telephone hotline was available for the patients until their next hospital visit for clinical examination.

Chondrogenic differentiation potential assessment

As the ethics committee evaluation advised against a second look arthroscopy in asymptomatic patients, we attempted to replicate the intra-articular environment by obtaining human cancellous bone

scaffold biopsied during the initial arthroscopy and incubating it with intra-articular hG-CSF activated autologous peripheral blood stem cells (AAPBSC) with Growth Factor Activation/Preservation (GFAP) concentrate and hyaluronic acid, to assess the mixture's chondrogenic differentiation potential.

Electron microscope photography and histological staining were performed and the results of one representative case are reported.

Results

Five patients (Table 1) underwent the AAPBSC harvesting, the IA injections, and the cancellous bone biopsy. AAPBSC harvesting was uneventful and without any side effects prior (period of 5 day hG-CSF stimulation), during or after the harvesting. The arthroscopy and subsequent injections on day 7 and 14 were also without incidents and apart from mild swelling and discomfort, no other complaints were noted. One month post-surgery, all pain medications could be withdrawn.

Cell population characteristics (Table 2)

The total nucleated cells of the harvested autologous activated peripheral blood stem cells ranged from 889.5 to 1,332x10³/L. The CD34 range was 0.34 to 1.04% and that of CD105 was 0.75 to 0.88% well within expected reference ranges and without any differences between fresh and post thaw samples. Cell viability of the fresh preparation was between 96.79

and 99.70%, while the post thaw specimens ranged between 67 and 86.66%.

Quality of life parameters

The WOMAC and KOO patient scores were improved throughout at one and six month follow-up compared to baseline on all patients (Table 3). One month post-surgery, all pain medications could be withdrawn.

Electron microscopy

In electron microscope scanning (Fig. 1, magnification at x2,000), the injected AAPBSC appeared to attach and proliferate on human cancellous bone scaffold.

Histology

In Fig. 2, the histological analysis on day 21 after incubation with AAPBSC, stained with toluidine blue demonstrated the existence of proteoglycan content while safranin O staining indicated glycosaminoglycan content (GAG) on the cell layer on the human cancellous bone scaffold.

Discussion

The present study clearly demonstrates successful clinical and laboratory results using the combination of intra-articular (IA) autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with

Table 1. Baseline patient characteristics

Patient	1	2	3	4	5	Statistic
Kellegan Lawrence Classification	2	2	2	2	2	2
Sex	Female	Female	Female	Male	Female	M:F = 1:4
Age (years)	59	57	58	54	58	57.20±1.92
Bodyweight (kg)	60	70	59	95	54.5	67.70±16.28
Height (cm)	145	160	165	173	162	161.00±10.22
Body mass index (kg/m ²)	25.29	27.34	21.67	31.74	20.76	25.36±4.46
Knee (side)	Right	Right	Right	Left	Right	R:L = 4:1
TF angle* (degree)	1° varus	2° varus	2° varus	0° varus	2° varus	1.20±0.84
Intraoperative lesion	Medial condyle	Medial condyle	Medial condyle	Patellofemoral	Medial condyle	-
ICRS classification	III	IV	III	IV	IV	-
Location	Medial condyle	Medial condyle	Medial condyle	Patellofemoral	Medial condyle	-
Size (cm ²)	2.5x2.5	2x2.5	3x2.5	2.5x3.5	2.5x3	7.00±1.43 (median 7.5)

Table 2. Autologous peripheral blood stem cell collection counts (total nucleated cells-TNC, CD34+, and CD105+ cells) and cell viability in all patients

Patient	Day	TNC x10 ³ /L in X ml	CD34 cells per 4 ml, as % of TNC		CD105 cells per 4 ml, as % of TNC		Viability (%)	
			Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
1	0	889.5	0.49		0.84		99.70	
	7	993.6		0.49		0.80		67.00
	14	1,014.8		0.49		0.80		77.24
2	0	996.0	0.54		0.88		99.40	
	7	1,266.8		0.54		0.82		72.11
	14	1,275.8		0.54		0.81		72.01
3	0	1,029.0	0.39		0.78		99.39	
	7	1,143.0		0.36		0.77		86.60
	14	1,236.5		0.34		0.75		73.54
4	0	1,056.0	1.04		0.85		96.79	
	7	1,129.3		1.03		0.82		85.45
	14	1,264.5		1.01		0.81		71.39
5	0	1,116.0	0.45		0.81		98.90	
	7	1,332.0		0.42		0.80		86.66
	14	1,325.3		0.41		0.78		71.82

Table 3. WOMAC and KOO patient scores preoperatively and at 1 and 6 month follow-up

WOMAC	Patient No. 1			Patient No. 2			Patient No. 3			Patient No. 4			Patient No. 5		
Time (month)	0	1	6	0	1	6	0	1	6	0	1	6	0	1	6
Pain	9	8	2	11	7	2	9	8	4	15	16	4	9	10	3
Stiffness	4	5	1	7	7	2	5	6	2	7	6	3	5	6	2
Function	35	28	7	43	33	15	40	32	18	45	41	14	32	28	14
Summary	48	41	10	61	47	19	54	46	24	67	63	21	46	44	19
KOOS															
Time (month)	0	1	6	0	1	6	0	1	6	0	1	6	0	1	6
Pain	15	13	8	14	13	7	15	14	8	20	16	10	14	13	7
Symptoms & stiffness	6	6	2	6	7	2	7	6	2	8	8	3	7	6	2
Function, daily living	12	16	4	14	17	5	17	17	8	19	22	12	12	16	6
Function, sports and recreational activities	12	16	6	15	14	7	12	18	9	16	16	8	14	18	7
Quality of life	10	12	5	12	12	4	10	13	7	12	15	6	8	12	6
Summary	55	63	25	61	63	25	61	68	34	75	77	39	55	65	28

hyaluronic acid (HA) in conjunction with arthroscopic microdrilling mesenchymal cell stimulation (MCS) in regenerating articular cartilage in early osteoarthritic knee disease that failed conservative treatment. Improved Quality of Life parameters (WOMAC and KOOS scores) were seen and one month post arthroscopy all pain medications could be withdrawn in all patients.

Preclinical studies in an OA animal model showed that bone marrow stem cells⁽¹⁰⁾ resulted in cartilage repair and peripheral blood stem cells in

humans⁽¹¹⁾ seem to contribute to articular cartilage regeneration following combinations of drilling and IA-HA. Similarly, intra-articular autologous adipose tissue derived stem cells and combinations of growth factors⁽¹²⁾ in a case series also showed encouraging results. The authors working hypothesis is that the combination of arthroscopic intervention and administered growth factors replicates the necessary articular environment and bypasses regeneration obstacles. The avascular and alymphatic nature of the

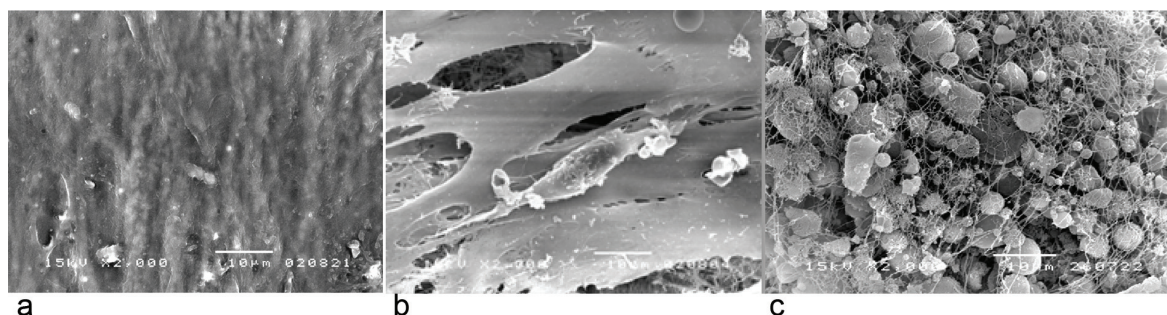


Fig. 1 Electron microscope (2,000 x magnification) human cancellous bone scaffold appearance of injected AAPBSC on day 21 of the incubation, showing stem cell attachment and proliferation (a) cancellous bone (b) attachment of the stem cell (c) population of stem cells.

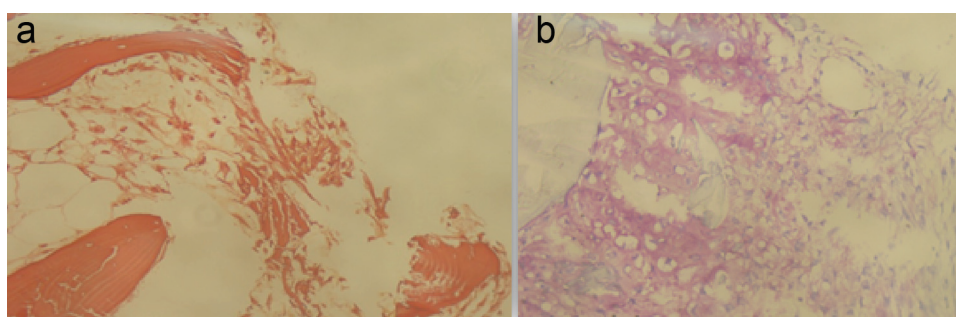


Fig. 2 Human cancellous bone scaffold histological appearance on day 21 of incubation with autologous AAPBSC and GFAP (a) Safranin O staining for glycosaminoglycan (GAG) and, (b) Toluidine blue staining for proteoglycans.

articular cartilage necessitates a scaffold that will successfully bring in the necessary nutrients and hold the operative cells in place to exert their effect whether that is fusion with local cells, stimulation and differentiation of local chondrocyte precursors, paracrine support, or a combination of all of the above. Arthroscopic microdrilling creates multiple points of initiation of cartilage regeneration by subchondral bone where blood clot acts as a natural scaffold to hold AAPBSC in place, aided by the HA. Autologous platelet rich plasma (PRP) may deliver crucial growth factors such as transforming growth factor (TGF) beta, insulin-like growth factor (IGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), all known to promote stimulation of bones, blood vessels, and chondrocyte formation⁽¹⁶⁻¹⁸⁾, which are also available in the authors preparation. The addition of articular hG-CSF further enhances cartilage regeneration by attracting additional bone marrow stem cells, aiding angiogenesis in an appropriate environment, and regulating the proliferation, migration, or differentiation of the adult mesenchymal stem cells⁽¹⁵⁾ locally. Moreover, the advantageous effects of hG-CSF in bone tendon

integration may extend to the subchondral area⁽¹⁵⁾. Furthermore, hG-CSF has recently been shown to attenuate the impact of ageing on bone marrow stem cells and recover age-related functional decline by significantly improving their proliferation activity and growth factor production such as NGF and BDNF both of which are found in the synovial membrane, are expressed in articular chondrocytes and play a role in knee arthritis⁽²¹⁾ thus bypassing age related limitations and objections in using autologous peripheral blood stem cells in cartilage repair⁽²²⁾. Moreover, our results also show that AAPBSC harvested through the minimally invasive procedure of leukapheresis contain significant numbers of mesenchymal CD105+ cells, adequate for cartilage regeneration, which bypasses the need for invasive bone marrow aspiration procedure with potential for complications and/or painful liposuction to obtain adipose stem cells tainted with potential safety issues.

As all our patients experienced substantial clinical improvements it was not deemed ethical to process with a second look arthroscopy and that was a condition for the ethical committee approval. The authors had therefore to devise a surrogate model to

measure chondrogenic differentiation potential and to elucidate the mechanisms behind it. Electron microscopy scanning revealed apparent cell attachment and evident proliferation on cancellous bone biopsy at day 21 while histological analysis on the same day, demonstrated that the cell layer on the cancellous bone biopsy showed increased contents of proteoglycans and glycosaminoglycans, indicative of hyaline cartilage presence. If a need for a second look arthroscopy arises in the follow up of any of the current or future patients, biopsies will be performed and reported.

Conclusion

The combination of intra-articular (IA) autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with hyaluronic acid (HA) in conjunction with arthroscopic microdrilling mesenchymal cell stimulation resulted in Quality of Life improvements measured by WOMAC and KOO scores and succeeded in regenerating articular cartilage in early osteoarthritic knee disease that failed conservative treatment. Further controlled studies are warranted to confirm the above results in larger groups and to elucidate the mechanism of hyaline cartilage regeneration.

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Potential conflicts of interest

None.

References

1. Scott RD. Three decades of experience with unicompartmental knee arthroplasty: mistakes made and lessons learned. *Orthopedics* 2006; 29: 829-31.
2. Steadman JR, Miller BS, Karas SG, Schlegel TF, Briggs KK, Hawkins RJ. The microfracture technique in the treatment of full-thickness chondral lesions of the knee in National Football League players. *J Knee Surg* 2003; 16: 83-6.
3. Kim IL, Mauck RL, Burdick JA. Hydrogel design for cartilage tissue engineering: a case study with hyaluronic acid. *Biomaterials* 2011; 32: 8771-82.
4. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res* 2000; 212-34.
5. Gooding CR, Bartlett W, Bentley G, Skinner JA, Carrington R, Flanagan A. A prospective, randomised study comparing two techniques of autologous chondrocyte implantation for osteochondral defects in the knee: Periosteum covered versus type I/III collagen covered. *Knee* 2006; 13: 203-10.
6. Moseley JB, O'Malley K, Petersen NJ, Menke TJ, Brody BA, Kuykendall DH, et al. A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med* 2002; 347: 81-8.
7. Turajane T, Tanavaree A, Labpiboonpong V, Maungsiri S. Outcomes of intra-articular injection of sodium hyaluronate for the treatment of osteoarthritis of the knee. *J Med Assoc Thai* 2007; 90: 1845-52.
8. Goldberg VM, Buckwalter JA. Hyaluronans in the treatment of osteoarthritis of the knee: evidence for disease-modifying activity. *Osteoarthritis Cartilage* 2005; 13: 216-24.
9. LaPrade RF, Bursch LS, Olson EJ, Havlas V, Carlson CS. Histologic and immunohistochemical characteristics of failed articular cartilage resurfacing procedures for osteochondritis of the knee: a case series. *Am J Sports Med* 2008; 36: 360-8.
10. Saw KY, Hussin P, Loke SC, Azam M, Chen HC, Tay YG, et al. Articular cartilage regeneration with autologous marrow aspirate and hyaluronic Acid: an experimental study in a goat model. *Arthroscopy* 2009; 25: 1391-400.
11. Saw KY, Anz A, Merican S, Tay YG, Ragavanaidu K, Jee CS, et al. Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid after arthroscopic subchondral drilling: a report of 5 cases with histology. *Arthroscopy* 2011; 27: 493-506.
12. Pak J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. *J Med Case Rep* 2011; 5: 296.
13. Tabit CJ, Slack GC, Fan K, Wan DC, Bradley JP. Fat grafting versus adipose-derived stem cell therapy: distinguishing indications, techniques, and outcomes. *Aesthetic Plast Surg* 2012; 36: 704-13.
14. Welte K, Platzer E, Gabrilove JL, Lu L, Levi E, Polivka A, et al. Purification to apparent

- homogeneity and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor. *Haematol Blood Transfus* 1985; 29: 398-401.
15. Sasaki K, Kuroda R, Ishida K, Kubo S, Matsumoto T, Mifune Y, et al. Enhancement of tendon-bone osteointegration of anterior cruciate ligament graft using granulocyte colony-stimulating factor. *Am J Sports Med* 2008; 36: 1519-27.
 16. Parsons P, Hesselden K, Butcher A, Maughan J, Milner R, Horner A. The biological effect of platelet rich-plasma on the fracture healing process. *J Bone Joint Surg Br* 2009; 91B (Suppl 2): 293-c.
 17. Li NY, Yuan RT, Chen T, Chen LQ, Jin XM. Effect of platelet-rich plasma and latissimus dorsi muscle flap on osteogenesis and vascularization of tissue-engineered bone in dogs. *J Oral Maxillofac Surg* 2009; 67: 1850-8.
 18. Wu W, Chen F, Liu Y, Ma Q, Mao T. Autologous injectable tissue-engineered cartilage by using platelet-rich plasma: experimental study in a rabbit model. *J Oral Maxillofac Surg* 2007; 65: 1951-7.
 19. Brittberg M, Peterson L. Introduction of an articular cartilage classification. *International Cartilage Repair Society Newsletter* 1998; 1: 5-8.
 20. Quintero M, Riera H, Colantuoni G, Khatib AM, Attalah H, Moldovan F, et al. Granulocyte-macrophage colony stimulating factor is anabolic and interleukin-1beta is catabolic for rat articular chondrocytes. *Cytokine* 2008; 44: 366-72.
 21. Grimsholm O, Guo Y, Ny T, Forsgren S. Expression patterns of neurotrophins and neurotrophin receptors in articular chondrocytes and inflammatory infiltrates in knee joint arthritis. *Cells Tissues Organs* 2008; 188: 299-309.
 22. Chiba Y, Kuroda S, Osanai T, Shichinohe H, Houkin K, Iwasaki Y. Impact of ageing on biological features of bone marrow stromal cells (BMSC) in cell transplantation therapy for CNS disorders: functional enhancement by granulocyte-colony stimulating factor (G-CSF). *Neuropathology* 2012; 32: 139-48.

การศึกษาความปลอดภัย และการรายงานผลการรักษาเบื้องต้น ของการรักษาข้อเข่าเสื่อมระยะต้น ที่รักษาด้วยวิธีการรักษาแบบดั้งเดิมไม่ได้ผลโดยการใช้เซลล์ต้นกำเนิดจากเลือดด้วยการเตรียมแบบการเก็บสารที่จำเป็นต่อการเจริญเติบโตร่วมกับ จี ซี เอส เอฟ และการฉีดไฮโดรเจน ไฮยาลูโรเนต (1,200 กิโลดาลตัน) โดยการส่องกล้องและกระตุ้นการสร้างกระดูกอ่อน

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ภูมิหลัง: การรักษาผู้ป่วยในโรคเกี่ยวกับข้อเสื่อมระยะต้นแบบอนุรักษ์นิยมที่ไม่ประสบความสำเร็จ ทำให้เกิดการผ่าตัดข้อเข่าตามมา ปัจจุบันมีการศึกษาเกี่ยวกับรักษากระดูกอ่อนที่เสื่อม โดยเซลล์ต้นกำเนิดจากไขกระดูกร่วมกับการใช้ไฮยาลูโรนิกเอซิดฉีด แต่ก็ยังคงมีข้อจำกัดคือในการเก็บเซลล์ต้นกำเนิดจากไขกระดูกเนื่องจากมีอาการเจ็บ ได้เซลล์ต้นกำเนิดที่จำนวนจำกัด จึงมีการใช้เซลล์ต้นกำเนิดจากเลือดร่วมกับการเจาะกระดูกผ่านกล้องและไฮยาลูโรนิกเอซิดพบว่าได้ผลการรักษาที่ดี แต่ยังมีข้อโต้แย้งในแง่ของลักษณะผิวกระดูกอ่อนที่เกิดขึ้นใหม่ รวมทั้งยังขาดการศึกษาขั้นพื้นฐานในด้านข้อมูลของการแสดงออกของจีน การเจริญเติบโตของเซลล์กระดูกอ่อน และการเกาะติดของเซลล์กระดูกอ่อน ด้วยเซลล์ต้นกำเนิดจากเลือดที่มีการเตรียมแบบการเก็บสารที่จำเป็นต่อการเจริญเติบโตร่วมกับ จี ซี เอส เอฟ และการฉีดไฮโดรเจนไฮยาลูโรเนต (ostetil, MW = 1.2 Mdal) โดยการส่องกล้อง และกระตุ้นการสร้างกระดูกอ่อน (growth factor preservation ร่วมกับ granulocyte colony stimulating factor) นับเป็นการศึกษาครั้งแรกในมนุษย์และยังไม่เคยได้รับการตีพิมพ์มาก่อน

วัตถุประสงค์: เพื่อรายงานผลการรักษาในเบื้องต้นในด้านความปลอดภัยและผลการรักษาในระยะเบื้องต้น ในโรคข้อเข่าเสื่อมระยะเริ่มต้นในผู้ป่วยที่รักษาไม่ได้ผลด้วยวิธีอนุรักษ์นิยม ด้วยเซลล์ต้นกำเนิดจากเลือดที่มีการเตรียมแบบการเก็บสารที่จำเป็นต่อการ

เจริญเติบโตร่วมกับ จี ซี เอส เอฟ และการฉีดโซเดียมไฮยาลูโรเนต (ostetil, MW = 1.2 Mdal) โดยการส่องกล้องและกระตุ้นการสร้างกระดูกอ่อน โดยรายงานการศึกษาความปลอดภัยและภาวะแทรกซ้อน ผลการรักษาและการแสดงออกของจิงจุกกายวิภาคของกระดูกอ่อนที่เกิดขึ้น และภาพถ่ายจากกล้องจุลทรรศน์อิเล็กตรอน

วัสดุและวิธีการ: ทำการศึกษาในผู้ป่วย 5 ราย แบ่งเป็นหญิง 4 รายและชาย 1 ราย อายุเฉลี่ย 56 ปี (52-59 ปี) ผู้ป่วยทุกคนได้รับการรักษาข้อเข่าเสื่อมด้วยวิธีอนุรักษนิยมแล้วไม่ได้ผล ผู้ป่วยจะได้รับการรักษา โดยได้รับการรักษาโดยใช้เซลล์ต้นกำเนิดจากเลือดร่วมกับการฉีดยาไฮยาลูโรนิกเข้าข้อเข่า และการส่องกล้องกระตุ้นกระดูกโดยฉีดยาจำนวนสามครั้งในห้องผ่าตัด และฉีดซ้ำอีกสองครั้งในสองสัปดาห์ ผู้ป่วยทุกรายจะได้รับการประเมิน WOMAC, KOO, EQ5D ก่อนทำการรักษา และภายหลังการรักษาที่ 1 เดือน และ 6 เดือน ระหว่างทำการผ่าตัดในครั้งแรกจะมีการเก็บตัวอย่างกระดูกจากข้อเข่าเพื่อนำไปทดสอบการเกาะติดของเซลล์ต้นกำเนิด การแบ่งตัวของเซลล์ต้นกำเนิด และลักษณะทางจุลกายวิภาคศาสตร์ของเซลล์ต้นกำเนิด รวมทั้งคุณสมบัติการแสดงออกทางจิงจุกของกระดูกอ่อน

ผลการศึกษา: ผู้ป่วยทุกรายไม่พบว่ามีผลแทรกซ้อนจากการรักษา มีผลการรักษาเป็นที่น่าสนใจพอใจโดยประเมินจาก WOMAC และ KOO score รวมทั้งมีการเปลี่ยนแปลงที่ดีขึ้นของ EQ5D ที่หนึ่งเดือน และหกเดือน และพบว่าหลังจากได้รับการรักษาไปแล้วหนึ่งเดือน ไม่มีผู้ป่วยรายใดที่ต้องรับประทานยาแก้ปวดเลย

จากการตรวจตัวอย่างกระดูกด้วยกล้องจุลทรรศน์อิเล็กตรอนจะพบได้ว่าการเกาะติดและเพิ่มจำนวนของเซลล์ต้นกำเนิด รวมทั้งมีการแบ่งตัวและการแสดงออกของเซลล์จากการศึกษาทางกล้องจุลทรรศน์ แสดงให้เห็นว่ามีการเกิดขึ้นของเซลล์เกาะติดกับกระดูก ที่บ่งว่ามีคุณสมบัติของเซลล์กระดูกอ่อน โดยพบว่าการเพิ่ม ของ SOX-9, collagen type II, aggrecan ซึ่งสามารถย้อมได้ด้วย toluidine blue และ safranin O ซึ่งบ่งบอกถึงการตรวจพบ glucosaminoglycan และ proteoglycan ในกระดูกอ่อน

สรุป: การศึกษาเบื้องต้นในด้านความปลอดภัยและผลการรักษาในระยะเบื้องต้น ในโรคข้อเข่าเสื่อมระยะเริ่มต้นในผู้ป่วยที่รักษาไม่ได้ผลด้วยวิธีอนุรักษนิยม ด้วยเซลล์ต้นกำเนิดจากเลือดที่มีการเตรียมแบบการเก็บสารที่จำเป็นต่อการเจริญเติบโตร่วมกับ จี ซี เอส เอฟ และการฉีดโซเดียมไฮยาลูโรเนต (ostetil, MW = 1.2 Mdal) โดยการส่องกล้องและกระตุ้นการสร้างกระดูกอ่อน มีความปลอดภัยและได้ผลดีในการรักษากระดูกอ่อนข้อเข่า รวมทั้งมีการแสดงออกของคุณสมบัติของเซลล์กระดูกอ่อนทางการแสดงออกของจิงจุก และความสามารถในการเพิ่มจำนวนและการเกาะตัว โดยภาพถ่ายจากกล้องจุลทรรศน์อิเล็กตรอน อย่างไรก็ตาม การศึกษานี้เป็นเพียงการศึกษาเบื้องต้น จำเป็นต้องมีการศึกษาต่อเนื่องในระยะยาวเพื่อประเมินผล