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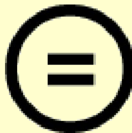

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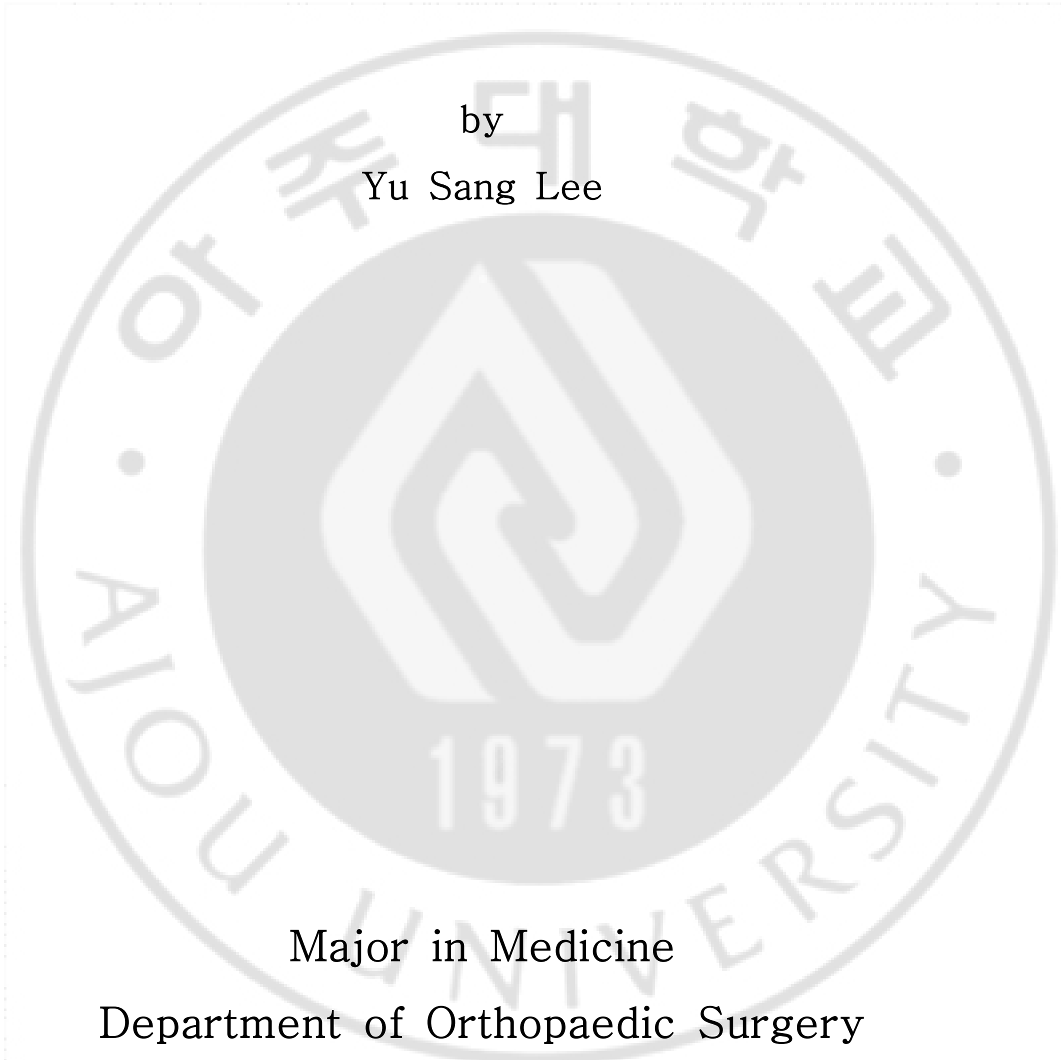
The Effect of Extracellular Matrix (ECM) Membrane Covering on Cartilage Repair using Microfracture and Abrasion Arthroplasty in the Rabbit Model

by

Yu Sang Lee



Major in Medicine

Department of Orthopaedic Surgery

The Graduate School, Ajou University

The Effect of Extracellular Matrix (ECM) Membrane Covering on Cartilage Repair using Microfracture and Abrasion Arthroplasty in the Rabbit Model

by
Yu Sang Lee

A Dissertation Submitted to The Graduate School of Ajou University
in Partial Fulfillment of the Requirements for the Degree of
Master of Medicine

Supervised by
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Major in Medicine
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Feburary, 2010

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- ABSTRACT -

The Effect of Extracellular Matrix (ECM) Membrane Covering on Cartilage Repair using Microfracture and Abrasion Arthroplasty in the Rabbit Model

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(Supervised by Professor Byoung-Hyun Min)

Marrow stimulating technique is first line treatment for focal cartilage defect, but scientific evidence for regneration mechanism was not investiated and restored tissue has weaker than normal hyaline cartilage. So, we investigate the colony frequency of fibrin clot obtained by marrow stimulation methods and the effect of preservation of fibrin clot in cartilage defect.

Colony frequency of fibrin clot obtained after microfracture and abrasion arthroplasty was counted. 24 rabbits were undertaken surgery (control, microfracture, abrasion arthroplasty) and extracellular matrix membrane was covered on defect area for half of them. Cartilage regeneration was estimated by histologic scoring system.

Colony frequency was increased as increasing bone marrow exposure area.

At 8 and 12 weeks after surgery, extracelluar matrix membrane covering groups revealed better result than no membrane covering groups.

Extracelluar matrix membrane covering after marrow stimulating technique is effective for cartialge defect. But further studies is needed.

Key Words: Cartilage defect, Microfracture, Abrasion Arthroplasty, Marrow stimulation technique, Extracellular Matrix Membrane

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I. INTRODUCTION

Articular cartilage defect rarely heal spontaneously regardless of whether the defects are acute, chronic, or degenerative in nature due to its avascularity and low cellularity(Frisbie et al, 2003; Dorotka et al, 2005). Usually, fibrocartilaginous tissue can be filled in full thickness defects that penetrate the subchondral plate; while healing is minimal or non-existent in partial thickness defects. These results may due to exposure of subchondral bone stimulate progenitor cells and growth factors which can induce healing process(Shapiro et al, 1993). In this point of view, surgeons have developed various marrow stimulation methods (e.g. drilling(Mitchell and Shepard, 1976), abrasion arthroplasty(Altman et al, 1992) and microfracture(Frisbie et al, 1999; Breinan et al, 2000; Frisbie et al, 2003)) intended to improve articular cartilage repair and decrease joint pain for patients with damaged articular cartilage or osteoarthritis (OA), and different outcomes resulted from each techniques(Menche et al, 1996).

However, none of these methods has shown restoration of a durable articular surface and it is unlikely that any of them will be uniformly successful in restoring articular surface(Dorotka et al, 2005). The reparative tissue is predominantly of a fibrous nature, containing variable numbers of chondrocytes, fibrocytes, and an unorganized matrix(Mitchell and Shepard, 1976). The fibrocartilage-like repair tissue that results from these techniques lacks the biomechanical and viscoelastic characteristics of normal hyaline cartilage and long-term clinical outcomes are unpredictable(Peterson et al, 2000).

To improve the quality of reparative tissue at the defect site, cultured autologous chondrocytes implantation (ACI) have been used in cartilage repair and autologous periosteal grafting has been investigated for containing cells in the lesion(Brittberg et al, 1994). Although excellent clinical outcomes have been obtained by this method, some complications (donor site morbidity, periosteal hypertrophy) were observed(Nehrer et al, 1999). Moreover, no significant difference was observed in

macroscopic and histological results between ACI and microfracture(Knutsen et al, 2004). Osteochondral autologous transplantations (OATS) are alternative method for cartilage defect, but can be used only smaller lesions because of limited availability of donor plug(Jakob et al, 2002).

In animal study, tissue engineered scaffolds have been developed to serve as a carrier for the implantation of cells into cartilage defect. R. Doroka et al. compared the effects of microfracture and scaffold for regeneration of cartilage. They have obtained good results treating full-thickness defects by microfracturing with chondrocytes transplantation using a collagen matrix(Dorotka et al, 2005). But such methods require a 2-stage operation and in vitro chondrocyte culture, therefore increasing the possibility of operative morbidity and alteration of chondrocytes phenotype(Darling and Athanasiou, 2005). J. Kramer et al. developed a 1-stage operation (penetrating subchondral bone and surfacing mesenchymal stem cell containing collagen I/III matrix at the same time) and made promising results(Kramer, Bohrnsen et al, 2006). That study introduced combination of marrow stimulation and tissue engineering.

Such methods are based on the hypothesis that exposure of bone marrow space can induce differentiation and proliferation of mesenchymal progenitor cell, but 'how many stem cells can be obtained and differentiated to chondrocyte' is still in question, although number, differentiation ability and preservation of progenitor cells are important for developing cartilage regeneration technique. We hypothesized that 1) microfracturing and abrasion arthroplasty can induce progenitor cells but number and differentiation ability of them are not same, 2) Preservation of fibrin clot in the chondral defect increase cartilage regeneration ability. So, we investigate the colony frequency of fibrin clot obtained by marrow stimulation methods and the effect of preservation of fibrin clot in cartilage defect.

II. Materials and Methods

All animal experiment protocols were reviewed and approved by AUSM-ERCAE (Ajou University School of Medicine - Ethics Review Committee for Animal Experimentation). All guidelines for procedures described in Animal Protection Law (Law4372, amended as Law 5153 and Law 5454) as administered by the Korea Department of Agriculture were kept strictly.

A. In vitro : Colony frequency and differentiation ability

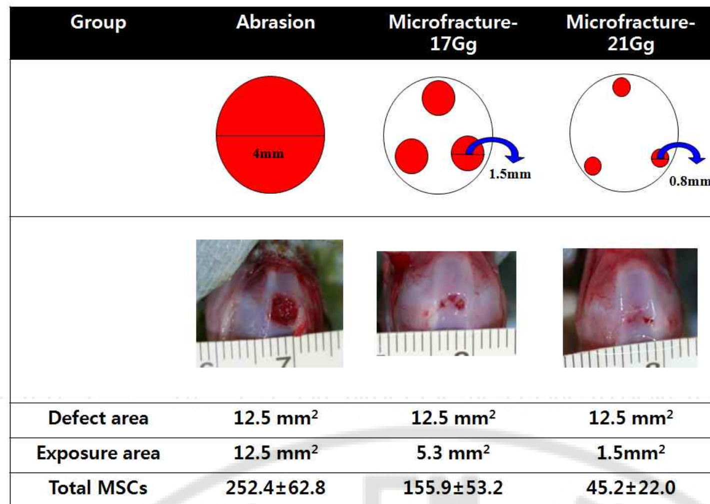
1. Animal Models

6 young New Zealand white rabbits weighting 3.5 Kg were prepared, and Zoletil® (Tiletamine + Zolazepam) 10 mg/kg and Rompun® 1 mg/kg were administered by intramuscular injection for anesthesia. Skin preparation (shaving, disinfection and draping) were carried out aseptically. Lateral parapatellar skin incision was made in both stifle joints of hind limbs of every animal followed by joint capsule incision and patella was dislocated medially for exposing trochlear groove.

Two groups of animal model were designed for comparing the colony frequency and effectiveness of progenitor cell harvesting. In the first group, cartilage defect size was equal for every defect (Group I), and in second group, subchondral bone exposure area was equal for every rabbit (Group II).

i) Group I (same defect size)

4mm sized full thickness cartilage defect were made in trochlear groove using biopsy punch (4mm diameter) and curette. 17 & 21 gauge needle and were used for microfracturing and abrasion was made by curettage of subchondral bone. 3 microfracture holes were made regardless of needle size(Fig. 1).



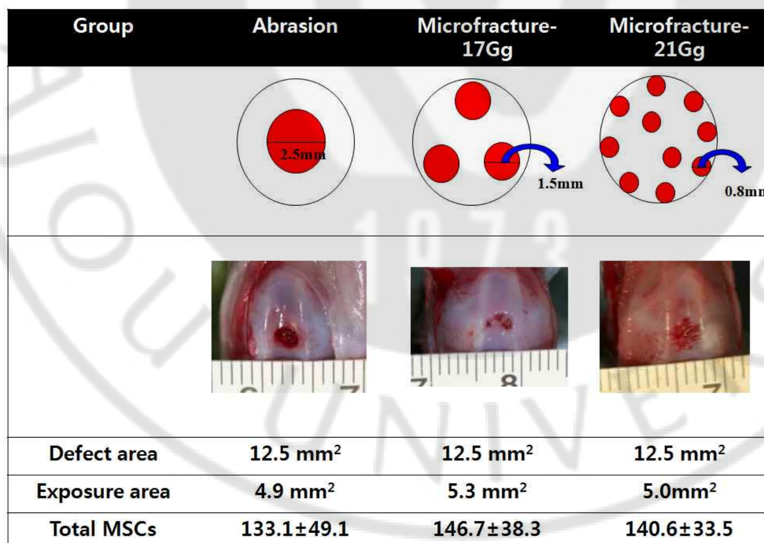
Group	Abrasion	Microfracture-17Gg	Microfracture-21Gg
Defect area	12.5 mm²	12.5 mm²	12.5 mm²
Exposure area	12.5 mm²	5.3 mm²	1.5mm²
Total MSCs	252.4±62.8	155.9±53.2	45.2±22.0

Fig. 1. In Vitro experiment for colony frequency in different treatment technique and different size of bone marrow exposure area

(Gg: Gauge, MNC: Mononuclear cell, MSCs: Mesenchymal stem cells)

ii) Group II (same subchondral bone exposure area size)

For same sized subchondral bone exposure, 3 holes for 17 gauge, 10 holes for 21 gauge and 2.5mm diameter abrasion arthroplasty were made. The size of removed subchondral bone was 5mm^2(Fig. 2).



Group	Abrasion	Microfracture-17Gg	Microfracture-21Gg
Defect area	12.5 mm²	12.5 mm²	12.5 mm²
Exposure area	4.9 mm²	5.3 mm²	5.0mm²
Total MSCs	133.1±49.1	146.7±38.3	140.6±33.5

Fig. 2. In Vitro experiment for colony frequency in different treatment technique and same size of bone marrow exposure area

2. Mononuclear cell culture and counting colony forming unit (CFU)

After subchondral bone exposure, Gelform® (4X4X2mm) was placed on the defect for collecting blood from subchondral bone for 2 min. Blood clot contained Geform® was incubated with 0.1% collagenase (Worthington Biochemical, Lakewood, NY, U.S.A.) mixed Dulbecco's modified eagles medium (DMEM: Gibco BRL,Grand Island, NY, U.S.A.) for 20 minutes (37°C, 5% CO2 incubator). Using a cell strainer (70 ㎛ Nylon, Falcon, Franklin Lake, NJ, U.S.A.), the cells were filtered, pooled, and centrifuged at 1500 rpm for 5 min. Washed twice with PBS, the cell pellet was resuspended in DMEM supplemented with 10% fetal bovine serum (FBS, Gibco BRL), 100 U/mL penicillin G (Gibco BRL) and 100 g/mL streptomycin (Gibco BRL). Mononuclear cell (MNC) number was determined using a trypan blue exclusion test. The cells were then plated at a density of 1.0 x 106 cells/cm2 on 6-well culture plate and placed at 37°C in a 5% CO2 incubator. The culture medium was changed 6th and 9th day after culture. At twelfth day, crystal violet stain was done for counting CFUs. Larger than 5mm diameter CFUs were counted and the colony frequency (CFUs / Seeded MNCs) was calculated.

B. In vivo : Membrane covering on chondral defect

1. Membrane preparation

We received ECM membrane (Artifilm®) from the cell therapy center of AUMC (Ajou university medical center, Suwon, Korea) for defect coverage. 8 mm biopsy punch was used for cutting the ECM membranes, because we need membranes larger than defect size. 8mm diameter ECM membranes were sterilized by EO gas sterilizer.

2. Animal Models

Six-months-old New Zealand white rabbits weighting 3.5 kg were prepared. Anesthesia and surgical approach were done same as in vitro model. To make

full-thickness chondral defect, a 5 mm diameter biopsy punch was used to line the defect and a 2 mm sized curette was used to extract the cartilage. To validate our full-thickness defect procedure, six rabbits were operated for rehearsal purpose.

3. Microfracture and Abrasion Arthroplasty

Twenty-four rabbits were prepared and divided into 3 groups (microfracture, abrasion arthroplasty and control) and each group consisted of eight rabbits(Table 1). The first group was received the microfracture treatment using 21 gauge awl on full-thickness defect on both knees until bleeding was observed. Five microfracture holes were made for each defect. After bleeding filled the defect and forming fibrin clot, the defect of right knee was covered by extracellular matrix (ECM) membrane and pasted using Histoacryl® (Enbucrilate, B.Brown medical AG, Switzerland). Another eight rabbits were received the abrasion arthroplasty treatment using a 2mm curette until bleeding was observed. Once again, the fibrin clot containing defect of right knee was covered by the ECM membrane(Fig 3). Control group received no treatment but every right knee was covered by ECM membrane. Joint capsule and skin was sutured layer by layer with carefully not to allow dislocation of patellofemoral joint. AluSpray® (Neogen®, MI, USA) was sprayed to protect operation wound against bacterial contamination. After operation, NSAIDs were administered for pain control and the rabbits were not immobilized and allowed activity in their cages until sacrifice.

Table 1. Experimental groups

	Control		Microfracture		Abrasion Arthroplasty	
Joint	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.
Membrane Covering	No	Yes	No	Yes	No	Yes

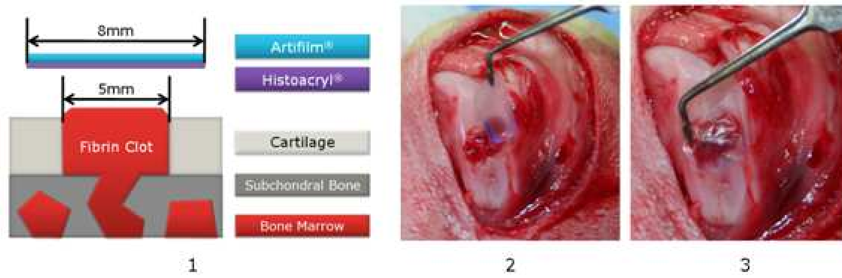


Fig 3. Scheme of ECM membrane covering on cartilage defect

4. Gross and Histological Analysis

At four and eight weeks after operation, two rabbits per each group were euthanized to observe healing process; the others were sacrificed at twelve weeks to elaborate healing results. Euthanasia was done by intravenous thiopental overdose injection after anesthesia by Zoletil®. The trochlear grooves were fixed for 48 hours in 10% neutral buffered formalin and decalcified in 5% nitric acid for 1 week in room temperature. For determining decalcification, decalcification solution was mixed with 5% ammonium oxalate. If mixed solution was remained clear, decalcification process was finished by neutralizing with 5% sodium sulfate for 12 hours followed by 70% ethyl alcohol for 12 hours. The decalcified samples were embedded in paraffin and sectioned at 4 μm. The sections were stained with hematoxylin and eosin (H&E) for overall structure, safranin-O stain for sulfated gycosaminoglycan, and immunohistochemistry for collagen type I and II.

Tissue types (articular cartilage, hyaline tissue, fibrous tissue, transitional tissue, bone) were determined as previously described criteria for cell and matrix appearance and presence of ECM component by histological staining(Breinan et al, 1997; Nehrer et al, 1998; Dorotka et al, 2005; Dorotka et al, 2005). For twelve weeks results, quantitative analysis of histology was done using O'Driscoll score(O′Driscoll et al, 1986) and Pineda score(Pineda et al, 1992). The degree and the quality of healing in all defects were assessed and scored blindly by two observers. The maximum possible score was 24 points in the O'Driscoll grading system (minimum 0 point) and 0 point in the Pineda scoring system (minimum 14 points). The reliability of both

scoring systems has been demonstrated in recent studies(Pineda et al, 1992; Moojen et al, 2002).

C. Statistical Analysis

Histologic scores of each group were analyzed with ANOVA method and statistical differences were checked. Colony frequency of In Vitro experiment was compared by T-test. SPSS 12.0.1 (SPSS Inc., Chicago, IL) was used for all statistical analysis.

III. Results

A. In vitro : Colony frequency and differentiation ability

Total count of mononuclear cells and CFUs of each defect were obtained and colony frequency was calculated. (Colony frequency = Total CFUs / Total mononuclear cell count)

1. Group I (same defect size, different size of bone marrow exposure area)

Two different marrow stimulation methods were done for each rabbit, in order to reduce variation of each rabbits. Abrasion arthroplasty did recruit more mononuclear cells and could make more colony forming units than microfracture groups. Between microfracture groups, 17G group was better than 21G(Fig 1).

2. Group II (same defect size, same size of bone marrow expose area)

Although different marrow stimulating techniques were used for exposing bone marrow, same size of expose area recruit same mesenchymal stem cells(Fig 2).

B. In vivo : Membrane covering on chondral defect

Six rabbits were tested to validate protocols for making full-thickness defect. During operation, no bleeding was shown in most of defects and minimal bleeding was controlled by compression of bleeding site. 1 week after operation, histological process was done and showed satisfactory results (data not shown). Most calcified cartilage was removed without bleeding and no reparative tissue was seen in defect lesion. ECM membrane was fixed by Histoacryl® and the glue and marrow-stimulated blood clot membrane showed chemical reaction and make hard covering material of the

defect, but no direct reaction to the defect surface was found.

All animals were survived the procedure without significant complication and resumed normal eating, drinking and activity habits within 24 hours.

C. Gross and Histological Evaluation

1. 4 weeks results

6 rabbits were sacrificed for 4 weeks results (n=2 for each group). Gross and histological analysis showed minimal healing processes in all groups(Fig 4). Some reparative tissue was found in treatment group, but not in control group grossly. Abrasion arthroplasty and microfracture showed no definitive difference grossly but abrasion arthroplasty made thicker tissue than microfracture.

Although membrane - glue mixture was found grossly, no membrane was observed microscopically in all groups and no glue reaction with bone and cartilage was found. ECM membrane covered groups didn't show better healing result until 4 weeks but represented more irregular surface and cartilaginous matrix than membrane uncovered groups.

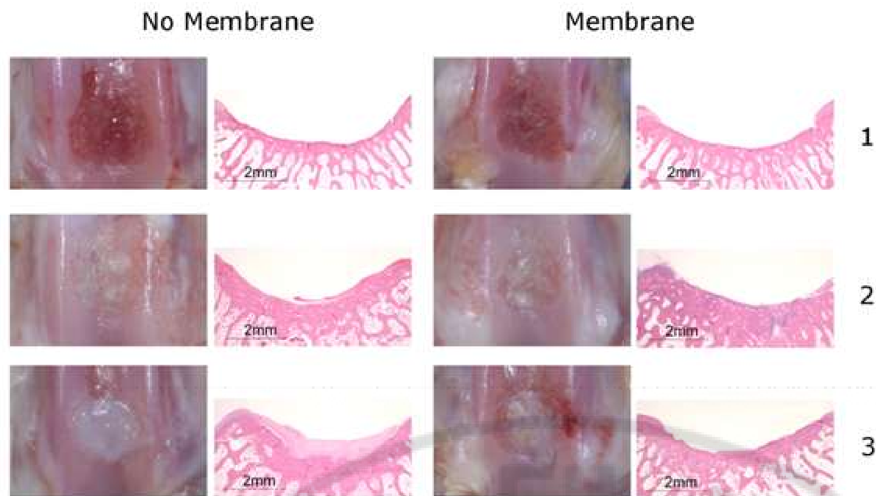


Fig 4. Gross and Histological specimens of the defect at 4 weeks

Gross and histological findings of defects at 4 weeks after operation. Control (1), microfracture (2) and abrasion arthroplasty (3) groups were observed by macro and microscopic methods.

2. 8 weeks results

6 rabbits were sacrificed to check healing processes at 8 weeks (n=2 for each group)(Fig 5). Compared to 4 weeks, thicker reparative tissue was formed in all groups and no treatment groups made healing tissue at 8 weeks. No treatment groups filled the defect with only fibrous scar tissue, but treatment groups produced more cartilage-like tissue, especially in membrane covered groups. In treatment family, membrane covered groups showed chondrocyte like cells in the lacunae and positive findings for proteoglycan in safranin-O stain, but membrane uncovered groups not detected. Hyaline-like cartilaginous tissue was found around microfracture hole in microfracture group, and in whole defect area in abrasion arthroplasty group.

Histoacryl® was remained in some areas and seems to be not degraded but surrounding healing tissue appeared same to those around tissue.

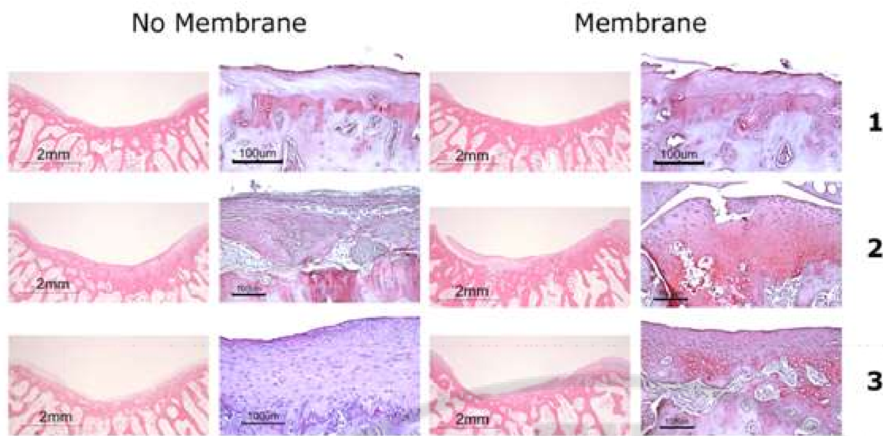


Fig 5. Histological sections of defects at 8 weeks

Histological findings of defects at 8 weeks after operation. Control (1), microfracture (2) and abrasion arthroplasty (3) groups were observed by microscopic methods after H&E (X20) and Safranin-O (X200) staining .

3. 12 weeks results & histological score

12 rabbits were sacrificed and examined grossly and histologically. Just like 8 weeks results, membrane covered groups showed better healing result than uncovered groups, and abrasion arthroplasty group's repaired tissue was most similar to normal hyaline cartilage(Fig 6). But, normal hyaline cartilage was not found in any reparative tissue, and subchondral bone sclerosis was found in all groups.

Qualitative histological score was obtained by using the O'Driscoll score and Pineda score. Sections from every cartilage defect areas were graded by 2 observers. In microfracture group, sections which didn't involve microfracture hole were selected for scoring. For each scoring system, ANOVA with Turkey HSD multiple comparison were tested. Significant between groups were 0.0004 in O'Driscoll score and 0.001 in Pineda score, and membrane covered groups were showed better results than uncovered-untreated group significantly(table 2,3). In treatment groups, membrane covering didn't make better different healing result than conventional methods

statistically, but membrane covering group's score showed less variation(Fig 7).

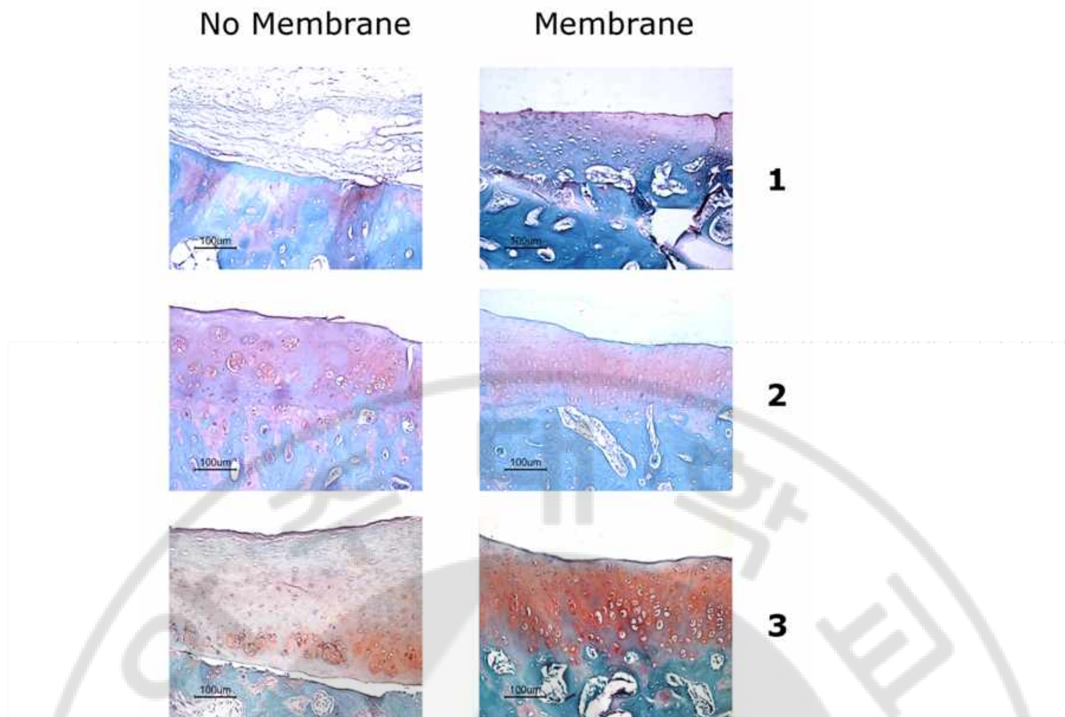


Fig 6. Histological sections of defects with Safranin-O stain at 12 weeks Histological findings of defects at 12 weeks after operation.

Control (1), microfracture (2) and abrasion arthroplasty (3) groups were observed by microscopic methods after Safranin-O (X200) staining

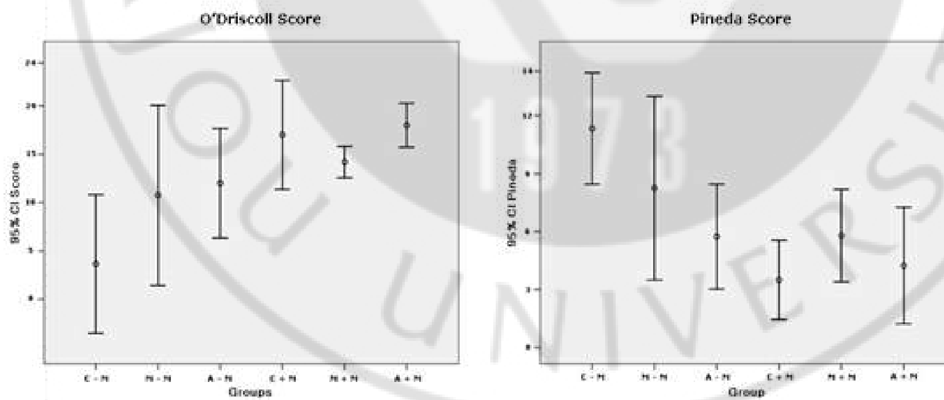


Fig 7. ANOVA for Histological Scoring System

Group		Mean Difference	Std. Error	Sig.
C - M	M - M	-8.333	2.598	0.047
	A - M	-10.533	2.484	0.006
	C + M	-7.083	2.598	0.118
	M + M	-14.333	2.598	0.000
	A + M	-13.333	2.598	0.001
M - M	C - M	8.333	2.598	0.047
	A - M	-2.200	2.282	0.923
	C + M	1.250	2.405	0.995
	M + M	-6.000	2.405	0.177
	A + M	-5.000	2.405	0.340
A - M	C - M	10.533	2.484	0.006
	M - M	2.200	2.282	0.923
	C + M	3.450	2.282	0.661
	M + M	-3.800	2.282	0.569
	A + M	-2.800	2.282	0.818
C + M	C - M	7.083	2.598	0.118
	M - M	-1.250	2.405	0.995
	A - M	-3.450	2.282	0.661
	M + M	-7.250	2.405	0.069
	A + M	-6.250	2.405	0.148
M + M	C - M	14.333	2.598	0.000
	M - M	6.000	2.405	0.177
	A - M	3.800	2.282	0.569
	C + M	7.250	2.405	0.069
	A + M	1.000	2.405	0.998
A + M	C - M	13.333	2.598	0.001
	M - M	5.000	2.405	0.340
	A - M	2.800	2.282	0.818
	C + M	6.250	2.405	0.148
	M + M	-1.000	2.405	0.998

Table 2. ANOVA for O'Driscoll score

C-M : Control without membrane, M-M : Microfracture without membrane,

A-M : Abrasion without membrane, C+M : Control with membrane,

M+M : Microfracture with membrane, A+M : Abrasion with membrane

Group A	Group B	Mean Difference (A-B)	Std. Error	Sig.
C - M	M - M	3.083	1.492	0.347
	A - M	5.583	1.492	0.016
	C + M	7.833	1.492	0.001
	M + M	5.533	1.427	0.012
	A + M	7.083	1.492	0.002
M - M	C - M	-3.083	1.492	0.347
	A - M	2.500	1.382	0.484
	C + M	4.750	1.382	0.030
	M + M	2.450	1.311	0.450
	A + M	4.000	1.382	0.086
A - M	C - M	-5.583	1.492	0.016
	M - M	-2.500	1.382	0.484
	C + M	2.250	1.382	0.592
	M + M	-0.050	1.311	1.000
	A + M	1.500	1.382	0.881
C + M	C - M	-7.833	1.492	0.001
	M - M	-4.750	1.382	0.030
	A - M	-2.250	1.382	0.592
	M + M	-2.300	1.311	0.516
	A + M	-0.750	1.382	0.993
M + M	C - M	-5.533	1.427	0.012
	M - M	-2.450	1.311	0.450
	A - M	0.050	1.311	1.000
	C + M	2.300	1.311	0.516
	A + M	1.550	1.311	0.839
A + M	C - M	-7.083	1.492	0.002
	M - M	-4.000	1.382	0.086
	A - M	-1.500	1.382	0.881
	C + M	0.750	1.382	0.993
	M + M	-1.550	1.311	0.839

Table 3. ANOVA for Pineda score

C-M : Control without membrane, M-M : Microfracture without membrane,

A-M : Abrasion without membrane, C+M : Control with membrane,

M+M : Microfracture with membrane, A+M : Abrasion with membrane

IV. Discussion

Many surgeons and researchers investigated articular cartilage and attempted to restore cartilage defect, so some operative techniques, such as ACI, microfracture, OATS, introduced and applied to patients. But, no methods could make regeneration tissue which has the same natural character of hyaline cartilage. ACI and microfracture are used widely, and have same short-term clinical and histological results(Knutsen et al, 2004). Microfracture is a kind of bone marrow stimulating technique, and makes mesenchymal stem cell contained fibrin-clot. Microfracture holes can anchor the fibrin-clot to cartilage defect, so this method showed better results than abrasion arthroplasty(Johnson, 2001). So, we contrived a simple method which can preserve marrow stimulated fibrin-clot in the lesion.

Rabbits are used broadly for cartilage defect animal model, because rabbit knee shows a gross morphological similarity to human knee joint and it is easy to purchase and feed(Rudert, 2002). Rabbit cartilage thickness is less than 400 μm, while the thickness of the human condylar cartilage is 2-3mm. Therefore, suture of ECM membrane to cartilage, as periosteal patch graft in ACI, is impossible. Attachment of ECM membrane is essential point of this study, Histoacryl® is used for membrane fixation. Histoacryl®, a member of the cyanoacrylate family, is a resorbable polymeric a powerful adhesive for tissues of high protein content like skin and tendon. It is not toxic, has a minimal inflammatory effect and does not interrupt tissue healing. It is used clinically and experimentally as an embolic and haemostatic agent in vascular surgery, tendon repairs, bone fixation in plastic surgery and osteochondral fracture fixation in orthopedic surgery(Yilmaz and Kuyurtar, 2005; Ayan et al, 2007).

Many materials have been used for defect covering(Chia et al, 2006; Kramer et al, 2006) and carrier of chondrocytes(Ting et al, 1998; Chaipinyo et al, 2004; Stevens et al, 2004; Gerard et al, 2005) and obtained promising results although their compositions are differ from native cartilage. Min and colleagues developed ECM membrane (Artifilm®, Cell therapy center, Ajou Univ. medical center, Suwon, Korea)

using xenogenic chondrocyte. Xenogenic scaffolds have been developed(Badylak et al, 2001; Badylak, 2004), but membrane type (like collagen membrane) ECM material had not been introduced. If the physical property of ECM membrane is strong enough to endure mechanical and chemical environment of joint space, it is better for healing process because it has natural components of cartilage those are not included in collagen or synthetic materials.

In this study, mesenchymal stem cells derived from subchondral bone by marrow stimulation methods were cultured and colony frequency was counted. If the defect area is same, abrasion arthroplasty showed higher colony frequency than other methods. This means that more mesenchymal progenitor cells can be recruited by abrasion arthroplasty, and may be helpful for cartilage regeneration. But, microfracture showed better healing results than abrasion arthroplasty in studies(Menche et al, 1996; Blevins et al, 1998), and theoretical benefit of microfracture was to enhance the attachment of repair tissue by way of the perforations into the bone(Johnson, 2001).

We hypothesized that if more MSC contained fibrin clot preserved in defect area, better quality of regeneration tissue can fill the defect. And with the aid of noble membrane, we covered the treatment area and obtained reasonable results. Although, O'Driscoll score did not show significant difference between membrane covered group and uncovered group, Pineda score revealed better filling potential of membrane covered groups, especially microfracture group. And standard deviation of membrane covered groups' histologic score was less than uncovered groups and abrasion arthroplasty and microfracture showed same regeneration ability in membrane covered groups. In another words, ECM membrane did good effect to cartilage healing process.

Variable kind of awls are used for clinical procedure of microfrature, but in our experiment, needle was used because, the defect is too small to use awl. So, different mechanism can be influence the healing process. But, we make holes with needle and mallet hammer, so some microfracture could be made and overall effect of procedure is regarded same for conventional microfracture and our procedure.

Limitations of this study are 1) small animal (rabbit) study, 2) small sized study (only 24 rabbits), 3) the safety of Histoacryl was not confirmed and direct effect of membrane to catrtilage was not known. So, large animal study and larger size study is needed and direct effect of membrane and Histoacryl (tissue glue) should be investigated.

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- 국문요약 -

토끼 모델을 이용한 미세절골술 및 소파 관절 성형술 후 세포외 기질막 첨가의 유용성

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(지도교수 : 민 병 현)

연골 결손 부위의 재생을 위해 골수 자극 수술법 (bone marrow stimulation technique)이 이용되고 있으나, 그 결과가 만족스럽지 않은 경우가 많아 수술 부위에 세포외 기질(Extracellular matrix, ECM)로 제작한 막을 덮어 재생의 효과를 높이고자 하였으며, 토끼를 이용한 실험을 통해 유용성을 알아보았다.

골수 자극법 시행 후 형성된 혈전에 중간엽 줄기세포의 발현 정도를 비교하기 위해 미세골절술과 소파관절성형술 후 획득한 혈전에서 colony frequency를 확인하였다. 또한, 24마리의 토끼를 대상으로 대조군, 미세골절술, 소파관절 성형술을 시행한 모델을 만들었으며, 세포외기질막을 덮은 군과 덮지 않은 군을 비교하였다.

colony frequency는 골수의 노출 정도에 비례하여 발현율이 높아지는 결과를 얻었다. 동물실험에서는 수술 후 8주와 12주후에 연골의 재생정도를 조직학적 분석을 하여 결과를 비교하였으며, 세포외기질막을 덮은 군에서 연골의 재생이 더 잘 이루어진 결과를 얻을 수 있었다.

본 실험을 통해 골수 자극 수술 후에 세포외 기질막을 덮는 것이 효과가 있음을 알 수 있었다. 하지만, 소동물을 대상으로 한 소규모의 실험으로 추가적인 실험이 필요할 것으로 사료된다.

핵심어 : 연골 결손, 미세골절술, 소파관절성형술, 골수 자극 기법, 세포외기질막