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COMPARISON OF MESENCHYMAL STEM CELL EMBEDDED WITH PEGDA SCAFFOLD AND MESENCHYMAL STEM CELL FROM BONE DRILLING TO REPAIR CARTILAGE INJURY

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Abstract: Regenerative medicine is a strategy of treat degenerative pathology. Mesenchymal stem cell (MSC) has been used as tool for tissue regeneration. Both MSC embedding in scaffold and MSC from microfracture techniques are presented by previous study for regeneration at site of cartilage injury. To studied and compare cartilage regeneration by MSC embedding in scaffold and microfracture techniques, we contributed a cartilages defect rabbit knee using a stainless steel drill. PEGDA-scaffold, which was embedded with MSC was implanted over the left knee lesion, whereas, the injured right knee was drilled deep into subchondral bone for leaking of bone marrow-MSC. Rabbits were sacrificed at 12 weeks after surgery to examine macroscopic appearance of the articular following the modified ICRS evaluation. Macroscopic appearance was evaluated by immunohistochemistry. We found that cartilage repairs with the uses of microfracture technique showed a better result as compared to the uses of MSC/PEGDA scaffold implantation technique, as seen in ICRs scores. The treatment with microfracture technique improved smooth cartilage surface, subchondral bone formation and reduce inflammation in rabbit knee. We conclude that microfracture technique is an efficient treatment for tissue regeneration in knee injury.

***Key words*: Histopathology, Knee injury, Mesenchymal stem cell, Scaffold**

1. INTRODUCTION

Articular joint injuries is one of the most difficult medical management due to the poor vascularization of the articular cartilage and the poor natural repairing process [1,2]. Various treatment modalities have been attempted but without satisfactory outcomes (3). Concept of combining the autologous cell-based therapy technology and the 3-D scaffold engineering hydrogels with have been resulted a promising outcomes in animal studies [4-9].

Treatment with bone marrow mesenchymal stem cell (MSC) has been well-accepted for tissue regeneration [8]. Bone marrow MSC is used for the treatment of cartilage defects [9]. Microfracture is a bone morrow stimulation technique, which is available bone marrow MSC to activate bone mesenchymal stem cells (MSCs) to migrate to cartilage defects where they can differentiate into chondrocyte and then achieve a cartilage defect [10].

The 3-D scaffold engineering hydrogels is used for MSC embedding and showed a result in successful articular repair. Many sources of MSCS, amniotic fluid and fetal umbilical cord Wharton Jelly (WJ) can be embedded in scaffold for cartilage tissue engineering. Many type of sponge scaffold were used such as poly (lactic acid) (PLA). PEGDA-agarose hydrogel is considered attractive materials to be encapsulated MSC for various tissue engineering. Kim et al.[11] found that MSC–embedded scaffold enhances the reparation of the rabbit knee joint injury while no cartilage repair.

2. MATERIALS AND METHODS

2.1 Cell culture and MSC/PEGDA scaffold encapsulation

MSC cell from Stem Cell Research and Development Unit, Department of Obstetrics & Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University. MSC at passage 8 were embedded in poly (ethylene glycol) diacrylate (PEGDA) hydrogel, which prepared from 10% (w/v) PEGDA (5000 Da, Jen Kem Technology, USA) and 0.2% (w/v) 2-hydroxy-2-methylpropiophenone (Sigma, USA), which served as a photoinitiator, in phosphate buffer saline (PBS). The preset solutions were exposed to UV light (365 nm) at an intensity of ~5.6 mW/cm2 for an entire duration of 60-480 s. MSC were encapsulated at a density of 7,000 cells/µl gel presets in a cylindrical mold with 4-mm diameter and 2-mm height. After exposed to UVA, the MSC/PEGDA scaffold was return to incubator for 16-18 h before used

2.2 Animal model manipulation

Ten male New Zealand white rabbits weighting over 3 kg were anesthetized using intramuscular injection of xylazine (1 mg/kg) and Zoletil® (5 mg/kg) and created Osteochondral defect using a stainless steel drill at 4-mm diameter and 2-mm depth in the center of patella groove in both knee joints under aseptic technique. The defect was washed with saline solution before implanting of MSC/PEGDA in to left knee. The right knee, which was made a defect by drilling deep in subchondral bone, was free from scaffold implantation. All rabbits were injected antibiotic and non-steroidal anti-inflammation agent for 7 days after surgery. The animal experimental protocol had been approved by Siriraj Animal Care and Use Committee (SI-ACUC), approval no. (COA No.003/2559, SiACUP No.SI-ACUP 015/2558)

2.3 Histological analysis

Rabbits were sacrificed at 4,8,12 weeks after surgery. Euthanasia were managed by overdose of anesthesia and potassium chloride. Both knee joints will be carefully opened. The surface of the distal head of the femur will be investigated. The sample collected were fixed with 10% formalin solution, decalcified with 10% nitric acid, imbedded in paraffin and cut into 3 µm slices before staining. Paraffin section were stained with hematoxylin and eosin (H&E) for histological evaluation. The cartilage defects were examined macroscopically for integrity and smoothness. Toluidine blue and safranin O were stained for proteoglycans observation. For immune-histochemical analysis, the collagen type I and type II were stained. All parameter were evaluated by a blinded pathologist using light microscopy (BX51; Olympus,Tokyo, Japan). The samples were also evaluated according the International Cartilage Repair Society (ICRS) visual histological scale modified by Mainil-Varlet et al [12].

2.4 Statistical analysis

All results were presented as means ± standard derivation (SD). Data analyses were performed using Student's t-test. A significant difference was evaluated by GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). A statistical significance was considered at a value of $P<0.05$..

3. RESULTS

3.1 Macroscopic result

All rabbits survived in the follow-up period of 12 weeks after surgery. No sign of wound infection or inflammation was observed. After total knee collecting, macroscopic defects were observed and morphologic appearance have been shown in Figure 1. At 4 weeks, the cartilages in the microfracture knee of all rabbits were covered with a glossy-white opaque tissue with almost complete of wound healing. In left knee of rabbit, which implanted with MSC/PEGDA scaffold, we found that cartilage surface was obviously presented wound defects and overlaid with blood clots. At 8 weeks, the wound of cartilage defect in the microfracture group was covered white opaque tissue. In the scaffold group, the defect was covered with whitish tissue in some areas, but the reddish area remained in center of the defect. The edge of defect got narrower. At 12 weeks, the cartilage defect in the microfracture technique showed covering of white opaque tissue. In the knee with MSC/PEGDA scaffold, the defect showed shallow along time increasing after surgery, with a cover of whitish tissue.

Figure 1. Macroscopic morphology of rabbit knee.
Femoral condyle of microfracture group and the MSC/PEGDA scaffold group at 4, 8, 12-week post-surgery are shown in figure (A, C, E) and (B, D, F) respectively.

3.2 Histological result

Histological appearances of cartilages were evaluated using hematoxylin eosin (H&E) staining and confirmed with assessment of toluidine blue and Safranin-O staining. The cartilage sections were examined using optical microscopy with a magnification of 40x. Histologic morphology was shown in Figure 2 (A, B, C). After implantation, our results showed that the sections of wound were initially closure by observation using microscope examination at 4 weeks in both groups studied. The dimension of defects was narrower in microfractured knee as compared MSC/PEGDA scaffold implanted knees throughout 4 to 12 weeks of study. Foreign body and signal of inflammatory was showed at high for tissue irritation in MSC/PEGDA scaffold implanted knees, but low signal in microfracture knees (Table 1). For zone assessment in hyaline cartilage formation, the result showed that cartilage in microfracture knee had an improvement of hyaline formation, whereas, hyaline cartilage formation was not found in all implanted knees. We found that hyaline formation was found in both group of knee at 12 weeks studied.

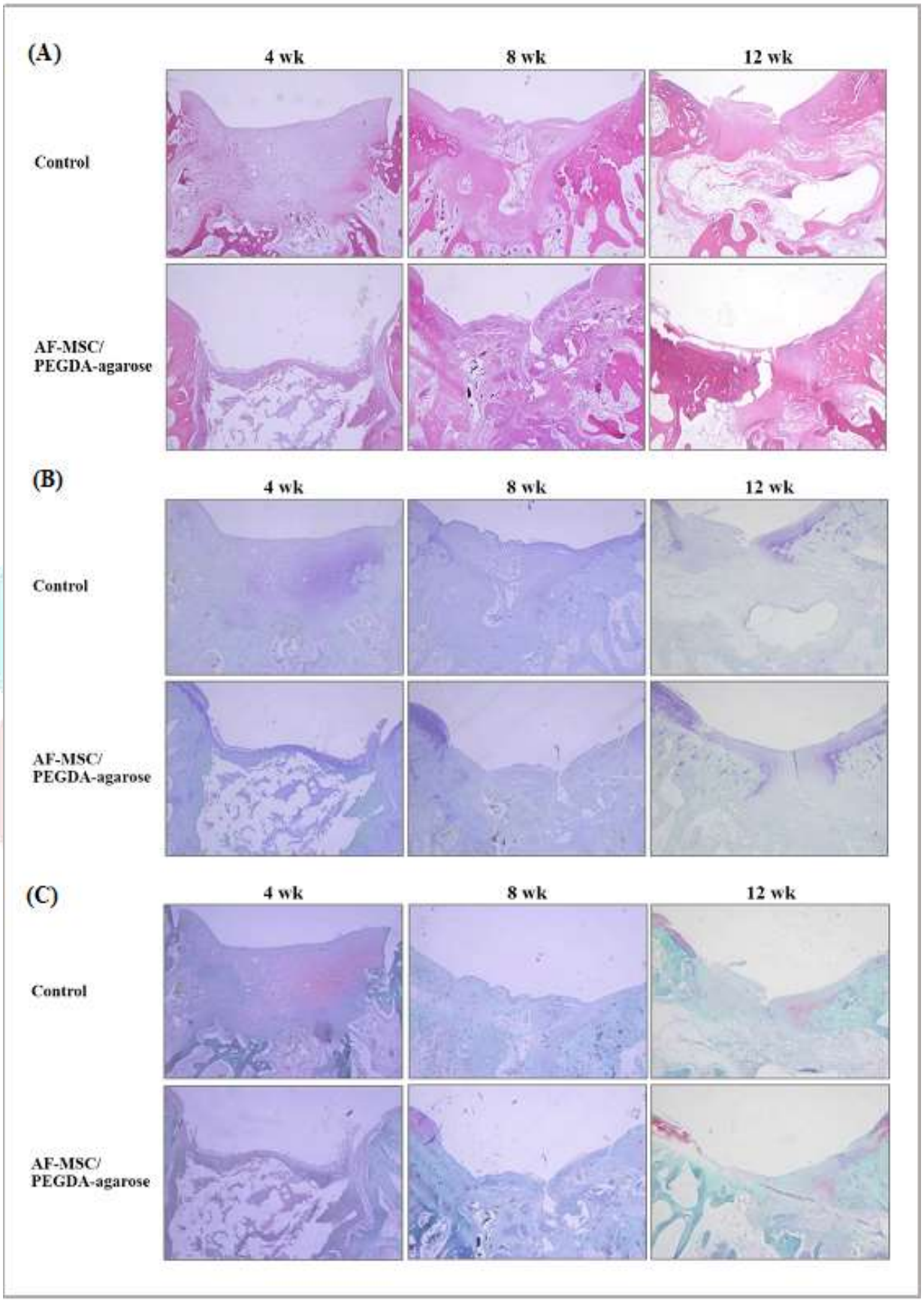

Figure 2. Histopathological appearance of cartilage formed.
Staining assessment were performed using (A) H&E staining, (B) toluidine blue staining and (C) safranin-O staining of the MSC/PEGDA scaffold implanted knee and microfracture technique of the same rabbit, at 40x magnification.

Parameter	Tissues	4 weeks	8 weeks	12 weeks
Dimension)width x depth(	MSC/PEGDA	3.17 x 3.17	4.17 x 4	3.13 x 3.9
	microfracture	3.8 x 1.93	3.37 x 2.03	3.33 x 1.6
Foreign body	MSC/PEGDA (n=3)	88.7%	77.3%	88.7%
	microfracture (n=3)	66.6%	0%	11.0%
Inflammatory lesion	MSC/PEGDA (n=3)	66.6%	77.3%	88.7%
	microfracture (n=3)	55.3%	0%	11.0%
Zone assessment for hyaline lining	MSC/PEGDA (n=3)	C,C,C	A,A,A	B,A,C
	microfracture (n=3)	A,A,B	B,A,A	A,C,A

Table 1 Macroscopic score after implantation

Score definition for zone assessment (A) deep zone, (B) middle zone, (C) not found

3.3 Scoring using modified ICRS score

In macroscopic evaluation, cartilages in microfracture knees were found a better score for improvement of smooth cartilage surface, subchondral bone formation, less formation of collagen type I as compared to cartilage in MSC/ PEGDA scaffold group. A significant difference of better result observed in microfracture were from scoring of matrix, cell distribution and viability of cell population. However, the knees, which were scaffold implanted, started to show a higher score of collagen type II expression at 8 weeks after implantation (Table 2). Microscopic observation of collagen type I and type II staining were presented in figure 3.

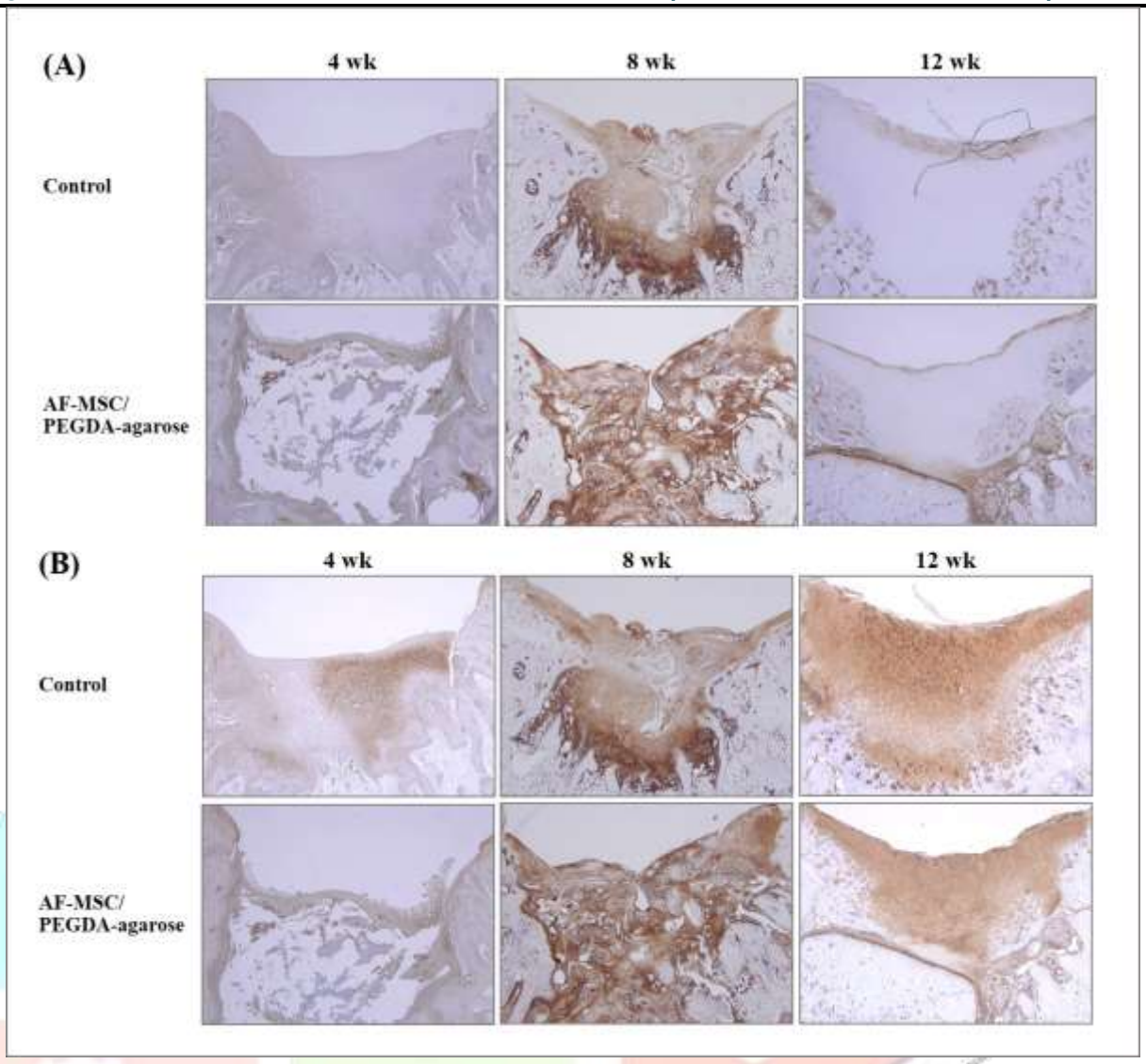

Figure 3. Immuno-histochemical staining of collagen type I and collagen type II

Histopathological appearance of rabbit cartilage after implantation for 4, 8, 12 weeks. Staining with Collagen type I antibody was presented in 3A, and collagen type II was presented in 3B. The presentation was performed at a magnification of 40x.

Table 2 Changes between groups according to modified ICRS visual histological assessment scale

Parameters	% of Group 4Wks		% of Group 8 Wks		% of Group 12Wks	
	microfracture knee	MSC/PEGDA knee	microfracture knee	MSC/PEGDA knee	microfracture knee	MSC/PEGDA knee
Surface						
Smooth/continuous	66.6 (2/3)	- (0/3)	- (0/3)	- (0/3)	- (0/4)	- (0/4)
Discontinuities/irregularities	33.3 (1/3)	100 (3/3)	1 00 (3/3)	100 (3/3)	100 (4/4)	100 (4/4)
***P*-value**	**0.1208**		**1.0000**		**1.0000**	
Matrix						
Hyaline cartilage	- (0/3)	- (0/3)	- (0/3)	- (0/3)	- (0/4)	25 (1/4)
Mixture:hyaline–fibrocartilage	100 (3/3)	- (0/3)	33.3 (1/3)	- (0/3)	75 (3/4)	25 (1/4)
Fibrocartilage	- (0/3)	33.3 (1/3)	- (0/3)	100 (3/3)	25 (1/4)	50 (2/4)
Fibrous tissue	- (0/3)	66.6 (2/3)	66.6 (2/3)	- (0/3)	- (0/4)	- (0/4)
***P*-value**	***0.0074**		**0.6401**		**1.0000**	
Cell distribution						
Mixed/columnar clusters	100 (3/3)	- (0/3)	33.3 (1/3)	33.3 (1/3)	75 (3/4)	- (0/4)
Clusters	- (0/3)	- (0/3)	- (0/3)	- (0/3)	- (0/4)	- (0/4)
Individual cells/disorganized	- (0/3)	100 (3/3)	66.6 (2/3)	66.6 (2/3)	25 (1/4)	100 (4/4)
***P*-value**	***<0.0198**		**1.0000**		***0.0242**	
Cell-population viability						
Predominantly viable	100 (3/3)	- (0/3)	66.6 (2/3)	33.3 (1/3)	75 (3/4)	- (0/4)
Partially viable	- (0/3)	33.3 (1/3)		33.3 (1/3)	- (0/4)	50 (2/4)
<10% viable	- (0/3)	66.6 (2/3)	33.3 (1/3)	33.3 (1/3)	25 (1/4)	50 (2/4)
***P*-value**	***0.0013**		**0.6433**		**0.0723**	
Subchondral bone						
Normal	- (0/3)	- (0/3)	- (0/3)	- (0/3)	- (0/4)	- (0/4)
Increased remodeling	66.6 (2/3)	- (0/3)	100 (3/3)	100 (3/3)	75 (3/4)	25 (1/4)
Bone necrosis/granulation tissue	33.3 (1/3)	66.6 (2/3)	- (0/3)	- (0/3)	- (0/4)	75 (3/4)
Detached/fracture/callus at base	- (0/3)	33.3 (1/3)	- (0/3)	- (0/3)	25 (1/4)	- (0/4)
***P*-value**	**0.1012**		**1.0000**		**0.6704**	
Cartilage mineralization						
Normal	- (0/3)	- (0/3)	- (0/3)	- (0/3)	- (0/4)	- (0/4)
Little	33.3 (1/3)	- (0/3)	- (0/3)	- (0/3)	- (0/4)	- (0/4)
Abnormal/inappropriate location	66.6 (2/3)	100 (3/3)	100 (3/3)	100 (3/3)	100 (4/4)	100 (4/4)
***P*-value**	**0.3782**		**1.0000**		**1.0000**	
Collagen type I						
Abundant	- (0/3)	- (0/3)	33.3 (1/3)	66.6 (2/3)	- (0/4)	25 (1/4)

Little	33.3 (1/3)	33.3 (1/3)	33.3 (1/3)	- (0/3)	50 (2/4)	25 (1/4)
None	66.6 (2/3)	66.6 (2/3)	33.3 (1/3)	33.3 (1/3)	50 (2/4)	50 (2/4)
***P*-value**	**1.0000**		**0.6433**		**0.5370**	
Collagen type II						
Abundant	33.3 (1/3)	- (0/3)	33.3 (1/3)	100 (3/3)	75 (3/4)	75 (3/4)
Little	66.6 (2/3)	33.3 (1/3)	66.6 (2/3)	- (0/3)	25 (1/4)	25 (1/4)
None	- (0/3)	66.6 (2/3)	- (0/3)	- (0/3)	- (0/4)	- (0/4)
***P*-value**	**0.1481**		**0.1155**		**1.0000**	

4. DISCUSSION

In animal studies, a rigorous study design includes histological characterization of the microfracture and scaffold implantation defect in an animal. Previous study presents an optimal time to treatment in rabbits is 2 to 6 months and 6 to 12 months as large animals studied [13]. As previously recommended, we setup our observation for cartilage repair in rabbits at 4, 8 and 12 weeks postoperatively. Animals were used to observed wound defect after bone microfracturing and MSC/PEGDA scaffold implantation. In our studied animal models, rabbit histological analyses can be supplemented with analyses of repair tissue via histological analysis. In cartilage repair models, histology of the knee joint can be used a ICRS scoring as a reference for intact cartilage volume and morphologic appearance. Intact pairing study between two treatments was established in same animal for osteochondral regeneration, which has been used in many previous study [10].

Microfracture technique is an approach to gain autologous bone marrow MSC from bone marrow by drilling into subchondral bone. Previous studies presented that autologous MSC was accepted for clinical applications in many countries [14-16]. Moreover, microfracture technique has been proved in several studies that can refill the site of injury defect [10]. In this study verified that microfracture is the one of strategy for knee injury treatment. However, autologous MSC which facilitated by microfracture is limited by the age of patient [17]. Another technique, using allogenic MSC is alternative opportunity for tissue regeneration. Although, the issue of risk in graft contamination, immunomodulation is on debate, allogenic MSC can be provided in a large number and obtained from various sources. The injection of allogenic MSC into knee injury site has been reported for effective outcome. Recent study of allogenic MSC implantation was developed into period of scaffold in order to docking MSC to the target site. Number of compatible scaffold were study. Our study investigated these two technique for cartilage regeneration in rabbit knees.

Despite numerous advances in the treatment of damage to the articular cartilage, an low cost, one-step approach to osteochondral defect repair. In order to address this need, a PEGDA-agarose hydrogel matrix scaffold has been designed within our group for osteochondral defect repair. This scaffold has previously demonstrated biocompatibility and the potential for cells to attach to injury site. The overall aim of this study was to assess the in vivo response of this scaffold and determine its potential to facilitate the repair of osteochondral tissue in rabbit knee. Specifically, we investigated the scaffold's ability to support host cellular infiltration and matrix deposition in vitro and in vivo if the layered arrangement of the scaffold led to tissue regeneration in a defect circumstance as similar to that of native osteochondral tissue. The study of macroscopic appearance in the articular surface was assessed using the ICRS I evaluation tool, compared two techniques of treatment. Histological analysis showed diffuse cellular infiltration and matrix production throughout the scaffold 12 weeks post-surgery. Although, improved repair was not observed in scaffold implanted groupas found in microfracture group, they demonstrated a high improvement of collagen type II formation using the modified ICRS histological scoring.

Stem cell treatment is an effective approach to improve cartilage regeneration. Form this data we conclude that microfracture is effective technique to treat knee injury, whereas, PEGDA-agarose hydrogel matrix might not be suitable scaffold for MSC embedding for tissue regeneration tools.

CONCLUSIONS

We conclude that microfracture technique is an efficient treatment for tissue regeneration in knee injury.

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CORRESPONDENCE

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ETHICAL STATEMENT

The study protocol was approved by the Siriraj Institutional Review Board, Mahidol University

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