Introduction:
Cartilage defects are hard to regenerate because the self-healing capacity of cartilage is limited. One of the most common techniques for repairing cartilage defects is microfracture arthroplasty that drills holes on the subchondral bone and draws out the bone marrow including stem cells to form blood clots and repair the cartilage defect. However, the repaired tissues by microfracture arthroplasty were mostly fibrocartilage rather than hyaline cartilage. We speculated that it is because the number of stem cells in blood clot is not sufficient or the blood clot is easily washed away by the knee motion and joint fluid. Bone marrow aspirates are currently widely investigated for the repair of various diseases including the cartilage defect. Mesenchymal stem cells (MSCs) in the bone marrow are thought to play an important role in its therapeutic effect. Based on the background, to find new treatment technique for cartilage injury, we isolated autologous marrow-derived buffy coat and injected to post-microfractured cartilage defect, and then used extracellular matrix (ECM) membrane to holding them.

Materials and Methods
Present study was conducted under the protocol approved by the animal experimental committee of Ajou University.
In vitro study: New Zealand white rabbits (weight: 3.0~3.5 kg) were used to isolate bone marrow from iliac crest or blood clots after microfracture on the cartilage defect. CFU-F assay was used to measure the frequency of MSCs in each sample.
In vivo study: Full thickness osteochondral defects were created in the trochlear groove of both knees of New Zealand white rabbits. The defect was remained untreated as negative control (Group 1), performed with microfracture only using 17 gauge needle (Group 2), injected with was remained untreated as negative control (Group 1), performed with microfracture arthroplasty (Group 3) and implanted with autologous osteochondral graft as positive control (Group 4). The cartilage defect was finally covered with a thin membrane made of cartilage matrix using cross suture method in groups 2 and 3. The repair of cartilage was evaluated by gross observation, histology, immunohistochemistry and chemical analysis for repaired tissue at 4, 8, and 12 weeks post-operation.

Results
The volumes of isolated bone marrow were 14 ul and 4 ml from blood clot of microfracture and iliac crest, respectively. The number of MSCs in the iliac crest bone marrow was 286 times higher than that in the blood clot by CFU-F assay (Table 1.). In the gross finding, the defect area showed smooth and glistening appearance and continuity with the surrounding host cartilage tissue during overall time in groups 3, and 4. In the histological observation at 4 weeks, the defect area were filled with fibrotic tissues in group 1, and fibro/hyaline tissues in the groups 2 and 3, while a hyaline-like cartilage was found in group 4. The content of hyalintissue was gradually increased with time in group 3, being significantly higher than those of groups 1 and 2. The ICRS score and the level of cartilage matrix proteins (type II collagen and GAG) also increased along with time more significantly in the groups 3 and 4 than in the groups 1 and 2 in the histological and immunohistochemical analyses(Fig 1).

Discussion
In present study, we showed that injection of autologous bone marrow buffy coat after microfracture arthroplasty effectively regenerated cartilage defect in rabbit model, being more effective than the microfracture arthroplasty alone and similar to autologous osteochondral graft. We speculate that the injected buffy coat could provide more MSCs to regenerate the defect and the ECM membrane covering the defect prevented injected cells and blood clots from leakage into the joint fluid. In conclusion, the injection of autologous bone marrow-derived buffy coat together with microfracture arthroplasty could be a useful clinical protocol for cartilage repair.

<table>
<thead>
<tr>
<th>Bone marrow of iliac crest</th>
<th>Cells/ul</th>
<th>Concentration/μl</th>
<th>MSC contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood clot of microfracture</td>
<td>39.25±9.8</td>
<td>15±5</td>
<td>8.54±1.18</td>
</tr>
<tr>
<td>Bone marrow of iliac crest</td>
<td>4000±500</td>
<td>15±5</td>
<td>2431.2±270.8</td>
</tr>
</tbody>
</table>

Table 1. MSC CFU-F assay

Fig 1. (A–D) Macroscopic observation, (E–H) Safranin-O/Fast green staining x20, (I–L) Type II collagen immune staining x20 at post operation 4 weeks.

References

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