Adhesive Barrier: Anisotropic Controlled Release for Cartilage Repair by Endogenous Progenitor Cell Recruitment

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Introduction: In this study, we investigated a new concept for implantable biomaterials to maximize the therapeutic effect should exhibit the following characteristics. First, the release direction of therapeutic proteins encapsulated within a hydrogel depot should be anisotropic. In other words, a direction of protein release from the microfracture site only to bone marrow is critical for effective autologous MSC migration to the disease site. Conventional depots release biomacromolecules in all directions. Second, loss of the migrated cells to the disease site should be prevented by providing a physical barrier with tissue adhesive properties. Implantation of the therapeutic depot (i.e. PDGF-AA loaded fibrin) is typical methods in tissue regeneration studies, but importantly, we developed an anisotropic ‘releasing-to’ approach by applying an adhesive gel patch (chitosan-catechol, CHI-C) on top of the fibrin gel, which plays a role in releasing the encapsulated PDGF-AA only to bone marrow direction for maximizing MSC cell migration. Furthermore, the migrated cells are retained by the adhesive chitosan-catechol barrier. We observed significant enhancement of therapeutic effect only when the ‘bone marrow-to’ anisotropic release and barrier functions are applied.

Methods: CHI-C gel patch was prepared by a freeze-dry process. Briefly, CHI-C (100 mg) was dissolved in 10 ml distilled and deionized water (DDW) and poured into the rectangular shape molds. CHI-C solution was frozen in refrigerator (- 20 °C) and then freeze-dried for 12 h. CHI-C barriers were stored in a desiccator before use.

Results: In vitro characterization of CHI-C gel patch for anisotropic release: CHI-C barriers were successfully inhibit the cell migration in the early stage. After 24 h incubation, CHI-C barriers were still blocking the cell migration. Thus, the CHI-C gel patch was expected to control the anisotropic release of macromolecules in depots and scaffolds, and to block the stem cell migrations. Thus, this model study for anisotropic release as well as spatial confinement of cell migration indicates that this new configuration can be useful for treatment of disease.

Dual in vivo imaging: In vivo bioluminescence imaging was performed in transplanted animals for 14 days. In the HCF-only Group without PDGF-AA (Group 1), the majority of the injected MSCs remained in the marrow cavity for 7 days. On the other hand, Groups 2 (plugged with PDGF-AA-loaded HCF) and 3 (plugged with PDGF-AA and TGF-β1-loaded HCF) showed a time-dependent movement of injected cells toward the osteochondral defect. Interestingly, Group 4, in which osteochondral defects were sealed with CHI-C barrier, showed faster and greater movement of injected cells than other groups. Also, chemotactic potentials of Group 4 were maintained for 14 days while the cell migration was stopped within 7 days in other groups. On day 14, all the signals in Group 4 congregated at the osteochondral defect. No signals were detected after day 14. Also, group 4 retained the greatest number of labeled MSCs cells. The patterns of imaging between fluorescence images of the postmortem femur and...
bioluminescence live images were similar, even though the detection methods of the signal were different from each other.

**Effect of migrated human MSCs on the healing of osteochondral defects:** Healing of osteochondral defect by the migrated MSCs was evaluated macroscopically and histologically. The macroscopic assessment demonstrated that the defect had become firm and smooth after 6 weeks in Groups 2, 3 and 4 while a visible defect was present in Group 1. The ICRS macroscopic score was highest in Group 4 (11.0), followed by Group 3 (9.3), Group 2 (8.3) and Group 1 (5.0). The histological findings from Safranin-O staining showed that the articular cartilage was well-regenerated in Groups 2, 3 and 4 with a smooth surface, abundant matrix and good integration with adjacent cartilage. However, the reconstitution of subchondral bone was better in Group 4 than in Groups 2 or 3.

**Tracking of migrated human MSCs in the regenerated cartilage:** While the migrated cells were rarely detected in Group 1, many fluorescent signals of the migrated MSCs were detected in the Groups 2, 3 and 4. The majority of the migrated MSCs were evenly distributed in osteochondral bone. But the fluorescent signals were not detected in the regenerated cartilage region. When the fluorescence positive cells were counted of the osteochondral bone region, 12-fold more human MSCs were detected in Group 4 than in Group 1 (p < 0.05) and it was 3.4- and 2.8-fold more than in Groups 2 and 3 (p < 0.05).

**Discussion:** We developed a novel system for osteochondral repair using anisotropic bio-adhesive gel patch and growth factor-loaded HCF in which the CHI-C barrier induced an anisotropic release of growth factors and blocked the dispersion of the migrated MSCs from the osteochondral bone region to other tissues. We confirmed that the CHI-C barrier enhanced the MSC migration to the osteochondral defect and induced better cartilage repair.

**Significance:** This system is expected to make a significant contribution in cartilage tissue engineering without cell transplantation.

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**Fig. 1**

![Image of experimental setup and results](image)