Regenerative tissue remodeling of a nude rat chondral defect model after transplantation of juvenile cartilage-derived chondrocyte sheets

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INTRODUCTION: Cartilage damage accounts for more than 12% of the causes of osteoarthritis, and there is an urgent need to establish treatment methods. Since cartilage has poor natural regenerative capacity and cannot be regenerated by existing therapies, many therapies using cells and biomaterials have been proposed. We have reported that human juvenile cartilage-derived chondrocyte (JCC) sheets can stably regenerate hyaline cartilage in nude rats 4 weeks after transplantation into chondral defects [1], and a clinical study of 10 patients with knee osteoarthritis has confirmed short-term safety and improved clinical scores [2]. We also reported that transplantation of effective donor cell sheets facilitates subchondral bone reconstruction at 4 weeks. However, the repair mechanisms of JCC sheets remain elusive. The purpose of this study was to elucidate the repair mechanism during the regeneration process of rat chondral defects by analyzing the changes over 4 weeks after cell sheet transplantation.
METHODS: JCCs determined to be effective in previous transplantation experiments were expanded from established cell banks and passage 2 JCC sheets were harvested. A focal chondral defect was created on the femoral patellar groove in nude rats and human JCC sheets were transplanted. Defect-only group was also prepared as a control. Animals were sacrificed after 1, 2, 3, and 4 weeks for histological evaluations (n=4-5 rats for each week). Human cell engraftment was confirmed with human-specific vimentin immunostaining. Safranin-O, COL1, and COL2 staining were performed to evaluate neocartilage quality. Proliferation cell nuclear antigen (PCNA) staining was performed to evaluate the proliferation of transplanted sheet and host cells at each time point. CD31 and osteocalcin (OCN) staining were performed to evaluate the angiogenesis and osteoblast activity. The animal study plan was evaluated and approved by Institutional Animal Care & Use Committee (IACUC, University of Utah) (assigned ID: 20-12001).
RESULTS: Representative histological samples of Safranin-O, COL1, and COL2 staining are shown in Figure 1. Safranin O and COL2 staining showed a gradual increase in positive cells, while COL1 staining finally reduced the number of positive cells at 4 weeks. In the defect-only group, safranin O and COL2 staining were negative for all weeks and COL1 staining showed positive cells in some areas. Representative time-course histological images of PCNA, CD31, and OCN staining are shown in Figure 2. PCNA staining showed actively proliferating cells in the transplanted cell sheets and in the host tissues at 1 to 2 weeks, followed by decreased proliferative cells at 3 weeks, and was almost absent at 4 weeks. In the defect-only group, a very limited number of PCNA-positive cells were observed at all time points. After JCC sheet transplantation, CD31-positive blood vessels and OCN-positive osteoblastic cells in the subchondral bone layer gradually increased up to 3 weeks, then dramatically decreased by 4 weeks. In the defect-only group, CD31 staining was negative at 1 week, then gradually increased from 3 weeks and the persistent blood vessels were observed at 4 weeks.
DISCUSSION: Dramatic morphological and phenotypic changes were found in human JCC sheets through 1 to 4 weeks after in vivo transplantation. It is noteworthy that COL2 expression occurred relatively early, but COL1 reduction, which is necessary for hyaline cartilage, is unlikely to occur before 3 weeks. The proliferation of chondrocytes within the transplanted cell sheet was found to occur 1 to 2 weeks after transplantation and be completed in 3 to 4 weeks. Based on these results, cell proliferation within the transplanted cell sheet was thought to occur early after transplantation, followed by mature differentiation by 4 weeks. Angiogenesis and osteoblast activity in the subchondral bone layer, which are important factors in subchondral bone reconstruction, occurred simultaneously from 1 to 3 weeks, then completed by 4 weeks. In contrast to the defect-only group, the early emergence of CD31-positive blood vessels in JCC sheet group were observed as early as 1 week, suggesting that the cell sheets may have stimulated host tissue angiogenesis early after transplantation.
SIGNIFICANCE/CLINICAL RELEVANCE: This study revealed the regenerative tissue remodeling within 4 weeks after transplantation of JCC sheets, which contributes to the elucidation of the action mechanism of cell sheet therapy in future clinical use.
REFERENCES: [1] Kondo M et al., npj Regen Med.2021;6(1):1-11. [2] Hamahashi K et al., npj Regen Med.2022;7(1):2-10.

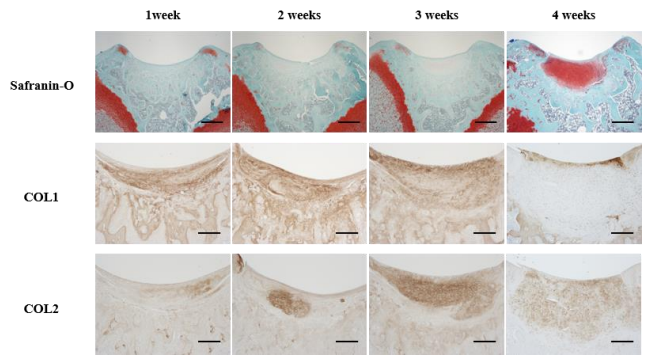

Figure 1. Representative histological samples of Safranin-O, COL1, and COL2 staining. Bars: Top rows: 500 μm, second and bottom rows: 200 μm.

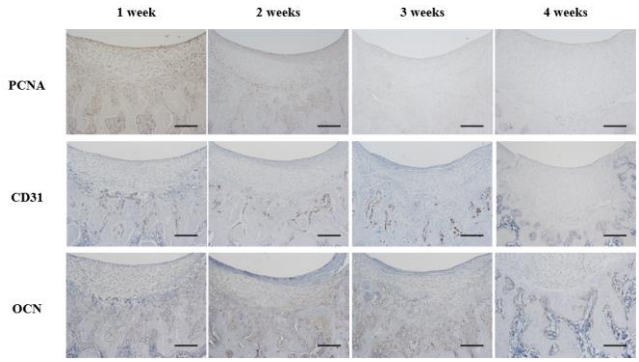

Figure 2. Representative histological samples of PCNA, CD31, and OCN staining. Bars: 200 μm