Study of the regenerative effects of xenogeneic transplantation in knee articular cartilage defects

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INTRODUCTION: While allogeneic transplantation has attracted much attention in regenerative medicine, animal models approximating safety and efficacy are continued to be explored in translational research because cell sources and transplantation modalities are diverse. Currently, we are preparing for a clinical study using allogeneic chondrocyte sheets for articular cartilage regeneration. In the present study, we investigated the regenerative effects of human chondrocyte sheets for articular cartilage regeneration in a xenogeneic transplantation model using immune deficient rats.

METHODS: Under the approval and guidance of the Tokai University Ethics Committee and with patients’ informed consent, articular chondrocytes and synoviocytes were obtained from patients (1 male, 1 female: average age = 73 years) undergoing total knee arthroplasty (TKA). They were co-cultured on temperature-responsive culture inserts (CellSeed Inc., Tokyo, Japan) for 2 weeks, and the chondrocyte sheets were then triple-layered and cultured for another 1 week as previously described1. Synoviocytes used as feeder cells were also recovered and pelleted for transplantation. An osteochondral defect on the patellar groove of the femur was created on one knee of each athymic nude rat (F344/Ncl-rmu/rmu; CLEA Japan, Fujinomiya, Japan). Four groups (each n=6) were created as follows: group A (untreated control); group B (chondrocyte sheet transplantation); group C (synovial pellet transplantation); Group D (chondrocyte sheet and synovial pellet transplantation). Rats were sacrificed at 4 weeks, and knee sections were evaluated histologically and scored using a modified version of the International Cartilage Repair Society (ICRS) grading system. Analysis of variance was used to analyze the ICRS scores, and the Tukey-HSD method was used for post-hoc testing. The results are expressed as mean ± standard deviation (SD), and p<0.05 was considered statistically significant.

RESULTS: In group B, defects were filled with cartilage-like tissue that stained for safranin O and type II collagen. In groups C and D, defects were filled with fibrous tissue that stained for type I collagen. In groups B, C, and D, parts of the repair tissue stained with anti-human vimentin antibody (Fig. 1). The ICRS scores were as follows: group A (19.4±3.1), group B (26.9±5.7), group C (21.5±1.9), and group D (22.5±5.6) with a significant difference between group A and group B (p<0.05).

DISCUSSION: In a previous experiment, we demonstrated that the combination of layered chondrocyte sheets and synovial pellet were more effective than only layered chondrocyte sheets or only synovial pellet in a rabbit allogeneic transplantation model1. Therefore, we expected that the combination of layered chondrocyte sheets and synovial pellet would promote better cartilage repair. However, repair was not observed in the current xenogeneic rat model. We suspect that differences in animal species or the viability of cells obtained from elder TKA patients may have been factors affecting our results. Previously, Shimizu et al. demonstrated in an allogeneic rat transplantation model that chondrocyte sheets but not synovial sheets promote cartilage repair and that the regenerated hyaline-like cartilage is largely composed of cells derived from transplanted chondrocyte sheets2. Takaku et al. demonstrated using luciferase expressing transgenic rat cells that transplanted cells survived in the defect and could be detected for more than 21 months in allogeneic transplantation3. Similarly, our results indicated that human chondrocyte sheet transplantation contributes to articular cartilage repair. Immunostaining also showed that much of the regenerated tissue comprised of cells of human origin after 4 weeks. This study has some limitations. The first limitation is that the immune response and in vivo environment is likely to be different between allogeneic transplantation and xenotransplantation. The second limitation is that only a histological evaluation was used, and in the future, functional evaluation is necessary for evaluating regenerated cartilage.

SIGNIFICANCE: This animal model is a useful tool in directly evaluating the safety and efficacy of human derived chondrocyte sheets in target location for treatment, namely in the knee joint environment.

REFERENCES:

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