Allogeneic transplantation of Scaffold-free constructs of adipose tissue-derived mesenchymal stem cells into tendon-bone junction of femoral bone tunnel in anterior cruciate ligament reconstruction in a rabbit model

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INTRODUCTION: ACL injury is a common sports injury and ACL reconstruction using hamstrings, quadriceps, or patellar tendon are generally performed for treatment. It is well known that successful ACL reconstruction depends on the secure healing of tendon-bone junction. Adipose-derived mesenchymal stem cells (ADSCs), which maintain pluripotency and self-proliferation ability, have received attention as a tool to enhance graft healing in ACL reconstruction [1]. Nakayama developed a novel method to create scaffold- free tubular tissue from multicellular spheroids using a "Bio-3D printer"-based system [2]. This system enables the creation of pre-designed three-dimensional structures using a computer robotics system. With this system, we created a tubular ADCSs constructs, and demonstrated the efficacy of ADSCs constructs to enhance the tendon-bone integration after ACL reconstruction [3]. Moreover, it is worth mentioning that allogeneic ADSCs allow the manufacturing of large batches of off-the-shelf ADSCs constructs, which would enhance the consistency and decrease the costs of cell therapy. So far, the potential concern of using allogeneic ADSCs constructs and the data of allogenic ADSCs constructs is limited. The aim of this study is to confirm the histological changes caused by allogeneic transplantation of ADSCs constructs into the tendon and bone junction in rabbit ACL reconstruction, and to confirm whether the transplantation is possible in the same way as autografting.
METHODS: [Study design] A total of 12 mature male Japanese white rabbits were used in this study. ADSCs were isolated from interscapular fat tissue in a rabbit and ADSCs constructs were prepared. Then, we performed rt. ACL reconstruction and implanted the allogeneic ADSCs constructs in the right femoral bone tunnel in 6 rabbits (implant group), and no implantation of ADSCs constructs was done in 6 rabbits (control group). We sacrificed 3 rabbits in each group at 3 and 6 weeks postoperatively. [Preparation of ADSCs construct] ADSCs were isolated from interscapular fat tissue in each rabbit, and expanded until the ADSCs spheroids were prepared. Spheroids were placed one-by-one in a microneedle array as programmed 3D data and cultured on the microneedles. Then Spheroids fused together and became one "ADSCs construct" which consisted of 240 spheroids obtained from 2 x 10^4 autologous ADSCs in a few days. After retrieved from microneedles, ADSCs constructs were cultured on tube for further maturation. (Fig.1) [Animal experimentation] We performed rt. ACL reconstruction. After the semitendinosus tendon was harvested, the knee joint was approached through a medial parapatellar approach, and the native ACL was transected. The tibial tunnel and the femoral tunnel were made at the anatomic footprint using 2-mm drill for tibia and 2.5-mm drill for femur. In implant group, when tendon graft was inserted into the right femoral bone tunnels, two pieces of tubular ADSCs constructs were inserted into the bone tunnel through the graft. (Fig.1) The graft was sutured to the periosteum in both knees with mild flexion. [Histological evaluation] Histologic assessment was performed using H&E and Picrosirius Red Stain to evaluate osteointegration and foreign body reactions of the tendon graft in femoral bone tunnel.
RESULTS: Macroscopic findings revealed no obvious signs of infection or findings suggestive of a strong foreign body reaction in either the implant group or the control group. Regarding histological evaluation, at 3 weeks, ADSCs construct was seen in the tendon-bone interface and migration of fibroblast cells were seen in the area between the bone and the construct in implant group. At the same time, the construct is mildly infiltrated with lymphocytes and eosinophils from bone side. In control group, fibrovascular tissue and cartilaginous tissue had appeared in the tendon-bone interface. At 6 weeks, collagen fibers and fibroblast cells, and cartilaginous tissue appeared in tendon-bone interface in implant group. The lymphocytes and eosinophils seen at week 3 had almost disappeared. In control group, fibrocartilaginous tissue was observed between tendon and bone and it was gradually blended into the tendon substance. (Fig.2, Fig.3).
DISCUSSION: We have demonstrated that autologous transplantation of ADSCs constructs promote bone remodeling at the interface of bone and tendon graft and enhances the bone tendon healing, biomechanically and biologically in previous research [3]. In this study, we demonstrated that allogeneic transplanted ADSCs constructs showed mild lymphocyte and eosinophil infiltration three weeks after surgery, but no strong foreign body reaction was observed. And it disappeared six weeks after surgery, and the tendon-bone healing was followed in a normal healing process. These findings could suggest that allogeneic transplantation of ADSCs constructs is possible in the same way as autografting. However, further studies are needed to verify that the allogeneic transplantation of ADSCs has positive effect on tendon-bone healing after ACL reconstruction in a rabbit model.
SIGNIFICANCE: Allogeneic ADSCs constructs created by bio-3D printer have potential to enhance the tendon-bone integration without strong foreign body reaction after ACL reconstruction.
REFERENCES: 1) Kosaka et al. Arthroscopy 2016, 2) Nakayama, Biofabrication 2013, 3) Higa et al. ORS annual meeting 2022,

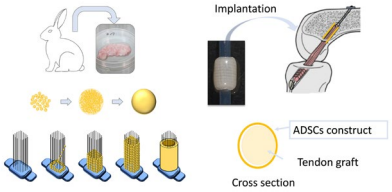

Fig.1 ADSCs constructs preparation

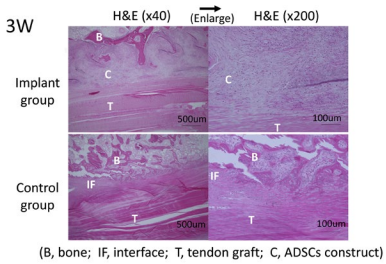

Fig.2. H&E staining of tendon-bone interface in femoral bone tunnel at 3 weeks after ACL reconstruction.

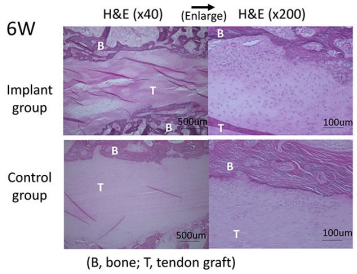

Fig.3 H&E staining of tendon-bone interface in femoral bone tunnel at 6 weeks after ACL reconstruction.