Establishment of a transplantable decellularized skeletal muscle for soft tissue defects

Shiro Fukuoka[1], Tomohiro Fujiwara[1], Kazuya Sawada[2], Takenori Uehara[1)], Masanori Yorimitsu[1)], Toshiya Fujisato[3], Toshifumi Ozaki[1]
[1]Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan.
[2]Department of Life Design, Osaka Seikei College, Osaka, Japan
[3]Department of Biomedical Engineering, Osaka Institute of Technology, Osaka, Japan
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INTRODUCTION:
Bone and soft tissue defect due to severe trauma or resection of malignant bone and soft tissue tumors often results in severe functional disability. In terms of bone defects after severe trauma or surgery for bone tumors, a variety of reconstruction techniques have been developed in these several decades; these include nonbiological reconstructions using custom-made or modular prostheses and biological reconstructions using allograft or irradiated or freezed autograft. However, the treatment choices for the large defects of soft tissue are limited; reconstruction of volumetric muscle loss commonly requires transplant of autologous muscle grafts, which present several drawbacks.
The drawbacks of transplant of autologous muscle grafts include insufficient donor tissue, particularly in case of severe injuries, loss of function, and high morbidity at the donor site. Alternatively, implant of biological biomaterials, composed of tissue's extracellular matrix (ECM), has been investigated. Decellularization protocols are tissue and organs dependent and directly impact the quality of the ECM. Ideal ECM-based scaffolds should mimic the structure, biochemical and biomechanical cues of the tissue to be reconstructed. This study aimed at identification of the ideal protocol of the methods on decellularization of rat skeletal muscles, which are an important tool for investigations in tissue engineering and translational medicine.

METHODS:
This study was approved by the Animal Care and Use Committees of the Okayama University.
Skeletal muscle decellularization. Decellularization of the tibialis anterior muscle of rats (male: around 300 g) was performed. A chemical method using sodium dodecyl sulfate (SDS) was conducted and optimal conditions for decellularization (SDS concentration and immersion time) were investigated. The quality of decellularization was evaluated by gross and stained images of the tissue sections and the amount of residual DNA.
Immunohistochemistry. Immunofluorescence was used to assess the presence of ECM components (laminin, fibronectin, and type I and type III collagen) in control and decellularized muscle. Primary anti-laminin antibodies (1:1000), anti-fibronectin (1:400), anti-collagen I (1:200) and anti-collagen III (1:100) were used. Alexa-Fluor 488 goat anti-mouse (1:500) was used as secondary antibody. Confocal microscopy was used for analysis.
Scanning Electron Microscopy (SEM). The surface morphology of the samples was observed with a scanning electron microscope (JSM-6390LM, JEOL Ltd.).The sample was fixed to an aluminum plate and gold-coated on the surface by sputtering equipment (SC-701AT, Sanyu Electronics Co., Ltd.) before surface observation.
DNA quantification. DNA quantification was performed using absorption spectrophotometry; the DNA concentration at which the absorbance at a wavelength of 260 nm as 1 was defined as 50 ng/ μ L.
Transplant experiments. The rat model of tibialis anterior muscle deficiency was used as the model for skeletal muscle defects. The space between the tibialis anterior and extensor digitorum longus muscles was expanded to create a defect of 7 x 10 mm in diameter in the muscle belly of the tibialis anterior muscle. Decellularized tissue was transplanted into the tibialis anterior muscle defect and fixed with fascial sutures. Tissue and mechanical evaluation was performed at 6 weeks.

RESULTS:
Optimization and characterization of decellularized skeletal muscle using sodium dodecyl sulfate. When decellularization of tibialis anterior muscle of rats was performed at several SDS concentrations (0.1%, 0.5%, 0.8%) with an immersion time of 1 day, HE staining revealed that nucleated cells remained in the muscle regardless of the SDS concentration, indicating that decellularization was insufficient with these conditions. Next, the immersion time was extended to 1 week, which resulted in no nucleated cells at all concentrations of SDS (0.1%, 0.5%, 0.8%). In these conditions, DNA quantification revealed that the residual DNA content in the decellularized muscle was less than 50 ng/μg, indicating that decellularization was successfully achieved. However, microscopical damages of the muscle tissue were observed at higher concentrations of SDS (0.5%, 0.8%). At low concentration of SDS (0.1%), no obvious tissue damage was observed.
Properties of the decellularized construct. Immunohistochemistry of decellularized tissue with 0.1% SDS showed residual ECM components. SEM of the decellularized skeletal muscle showed the presence of hollow annular structures, but the myofibers were removed.
Transplantation of the decellularized skeletal muscle. Decellularized rat anterior tibialis muscle was allogeneically transplanted into anterior tibialis muscle-deficient rats, and tissue evaluation was performed at 1, 2, 3, and 6 weeks after transplantation. Gross findings confirmed regeneration of the transplanted area at 3 weeks after transplantation, and histological evaluation at 6 weeks after transplantation confirmed that the transplanted area had been replaced by muscle tissue.

DISCUSSION:
Tissue decellularization is performed by chemical, physical, or enzymatic methods, or a combination thereof. We used the clinically applied chemical method with SDS, which takes advantage of its properties to solubilize cell membranes and separate DNA from proteins (1). SDS is a relatively strong detergent and causes matrix denaturation when used at high concentrations and for long periods of time (2, 3).It is important to establishe a protocol of decellularization which guarantees cell removal but minimize deleterious effects to the ECM physical structure and composition in order to maintain biological function of the skeletal muscle.
Previous studies suggested a decellularization protocol with mincing the muscle and then immersing it in a high concentration of SDS. However, these results indicated considerable damage to the skeletal muscle tissuse. In this study, decellularization of skeletal muscle was possible with a simple protocol: prolonged immersion in a low concentration of SDS resulted in success in maintaining skeletal muscle morphology. This method resulted in residual ECM and suppressed structural destruction of skeletal muscle tissue. The maintenance of skeletal muscle function would be great interest, which is our next step.

SIGNIFICANCE/CLINICAL RELEVANCE:
This study demonstrated the successful protocol in decellularizing skeletal muscle remaining abundant myofiber structures. Trasplantation of the decellularized muscle graft under our protocol may be a novel option for reconstruction of the large soft tissues in the treatment of severe trauma or bone and soft tissue tumors.

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