

Tissue Engineered Skeletal Muscle Using Recellularized Whole Muscle

INTRODUCTION: For the reconstructive surgeon, tissue engineered skeletal muscle may offer reduced donor site morbidity and an unlimited supply of tissue. This work demonstrates a novel technique for preparing functional tissue engineered muscle.

METHODS: Muscles from adult rats were acellularized using a protocol developed in our laboratory. These acellular muscles were placed in a bath of 20% fetal bovine serum in DMEM and 100 U/mL penicillin for one week at room temperature. C2C12 cell line myoblasts were used to repopulate the acellularized muscles. The cells were injected into the acellular muscle matrix using a 26-gauge needle and a 100 microL syringe. The resulting constructs were placed in growth medium (DMEM, 20% fetal bovine serum, and 100 U/mL penicillin) for one week at 37 deg C under 5% CO₂, with media changes every 48 hrs. The constructs were then placed in differentiation medium (DMEM, 6.5% horse serum, and 100 U/mL penicillin) for one week, with media changes every 48 hrs. Isometric force output and excitation-contraction properties of the constructs were determined. The constructs were then sectioned for histologic analysis.

RESULTS: The repopulated constructs produced longitudinal contractile force on electrical stimulation. The average peak force (Po) was 5.8 microNewtons, for a specific force (defined as peak force divided by cross sectional area) of 0.12 kN/m². Histologic sections demonstrated that the acellularized muscle was without nuclear staining, while the repopulated constructs had peripheral nuclear staining.

CONCLUSIONS: The resulting tissue engineered muscle constructs were able to produce small amounts of longitudinal contractile force. Further directions of study include development of larger, more robust constructs, and the incorporation of a vascularized pedicle.

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