ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

3D-bioprinted in vitro skeletal muscle with pennate fiber architecture to enhance contractile function

Supplementary File

Fluorescence staining

The expression of F-actin in differentiated cells of the muscle tissues was assessed by fluorescence staining to characterize the differentiation rate of C2C12 cells. Alexa Fluor 488 Phalloidin and 4', 6-diamino-2-phenylindole (DAPI) were used to label F-actin and the nucleus, respectively. For the convenience of staining and observation, the tissues were cut into thin pieces. The slices were washed twice with phosphate-buffered saline (PBS) preheated at 37°C, and fixed with Immunol Staining Fix Solution at room temperature for 10 min, then washed 2–3 times with PBS, with each wash lasting 10 min. The pieces were then permeabilized with 0.5% Triton X-100 solution for 5 min, and washed with PBS 2–3 times for 10 min each wash. The samples were incubated in Alexa Fluor 488 Phalloidin (1:200) at room temperature in the dark for 30 min. After incubation, the samples were washed with PBS three times, with 5 min for each wash, followed by incubation with DAPI solution (100 nM) for 30 s. After washing, fluorescence observation was conducted under confocal laser scanning microscopy (CLSM).

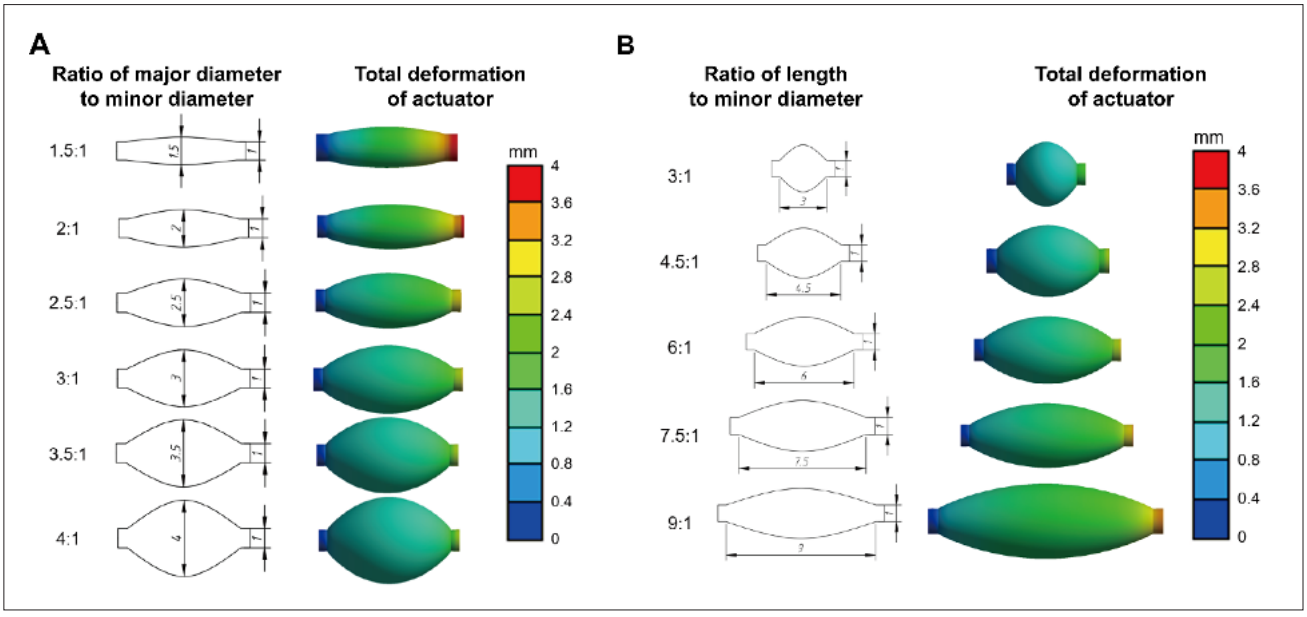

Figure S1. Simulation results of the macro shape of muscle model. (A) Modeling and mechanical simulation of pennate muscles with different ratios of major diameter to minor diameter at fixed minor diameter (1 mm) and length (6 mm). (B) Modeling and mechanical simulation of pennate muscles with different ratios of length to minor diameter at fixed minor diameter (1 mm) and major diameter (3 mm).

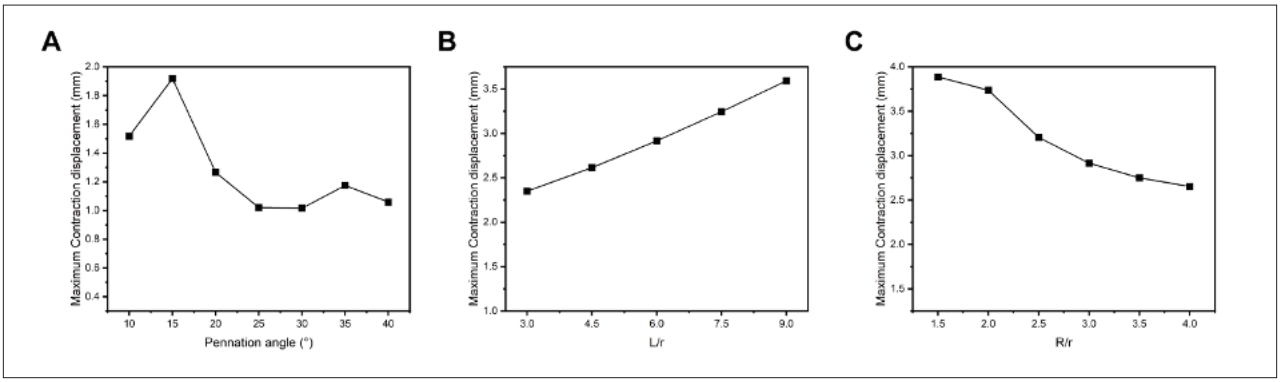

Figure S2. Contraction displacement of the tissue models with different (A) pennation angles, (B) the ratio of length *L* to minor diameter *r* (*L*/*r*), and (C) the ratio of major diameter *R* to minor diameter *r* (*R*/*r*) in simulation experiments.