

RESEARCH ARTICLE

# Preparation and characterization of angled dual- and multi-branched nerve guidance conduits

## Supplementary file

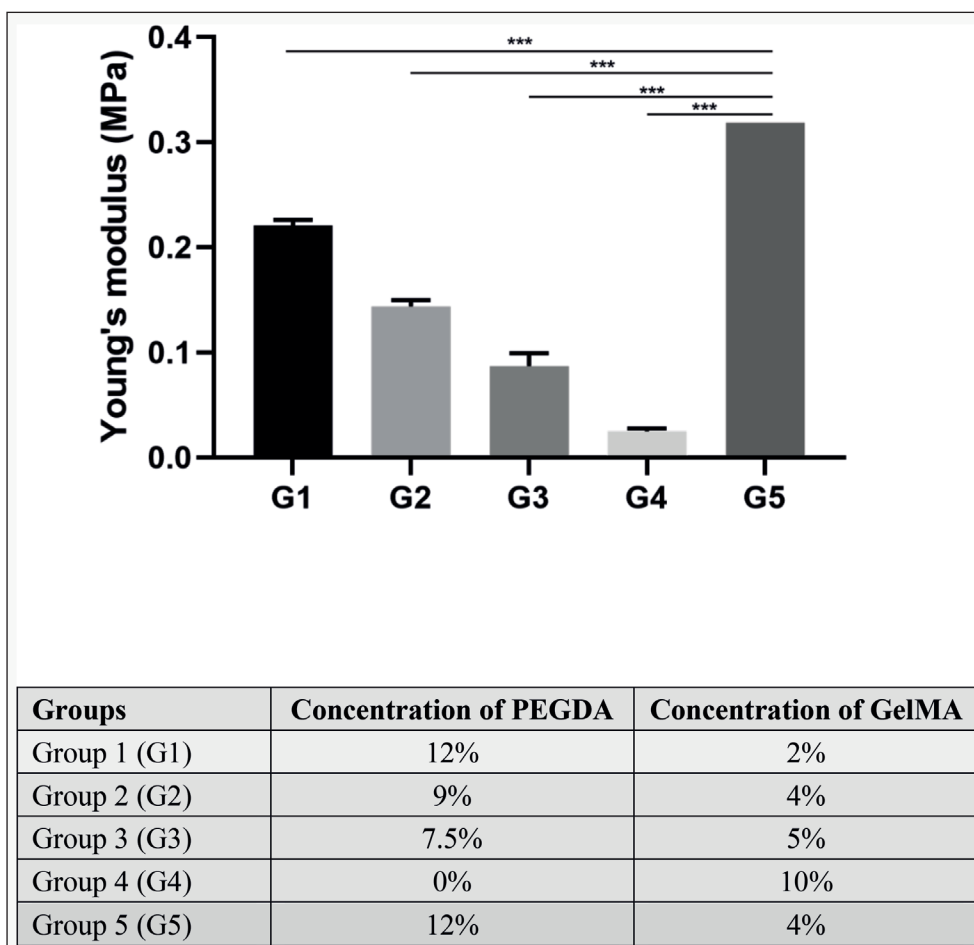
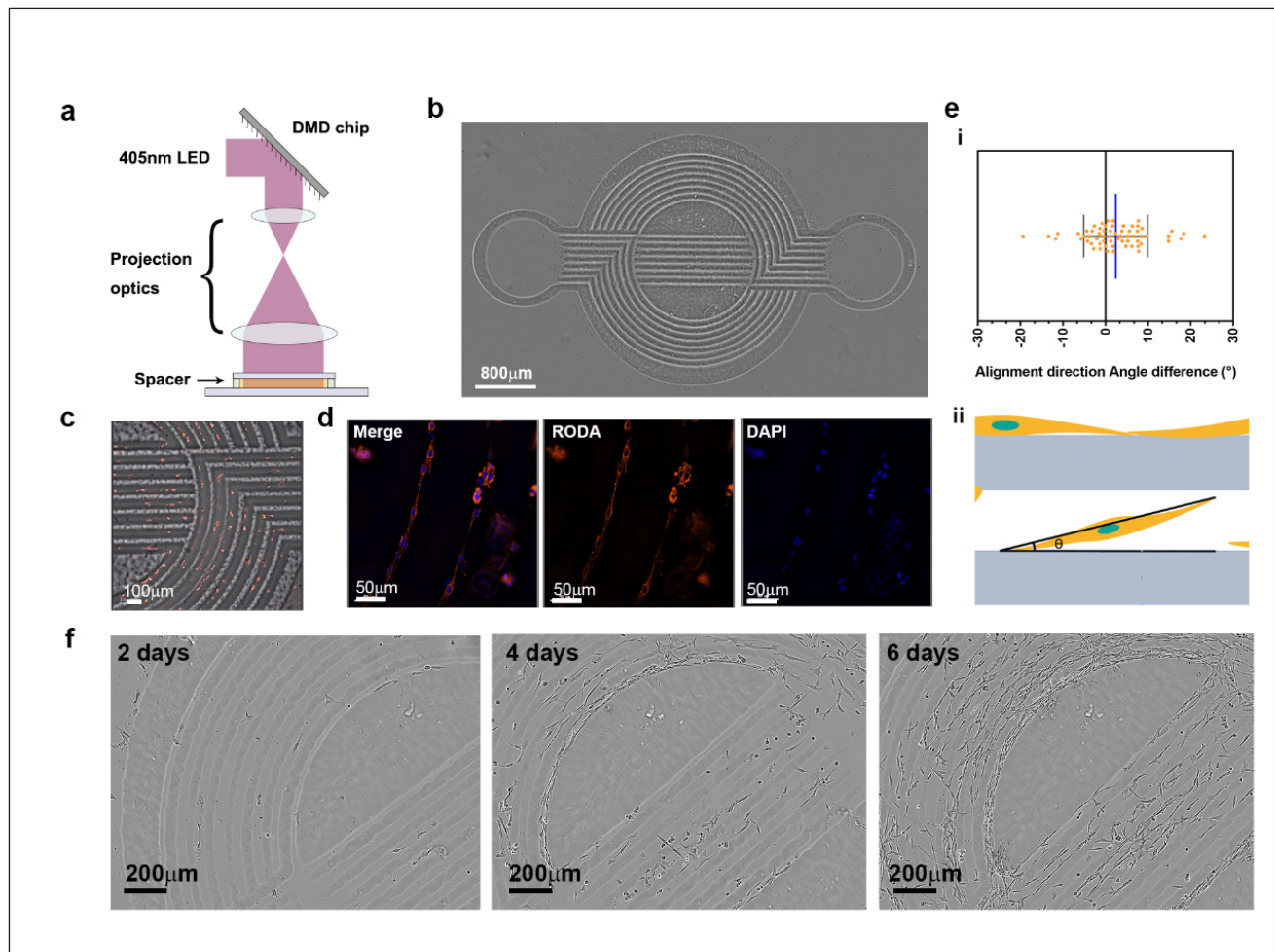
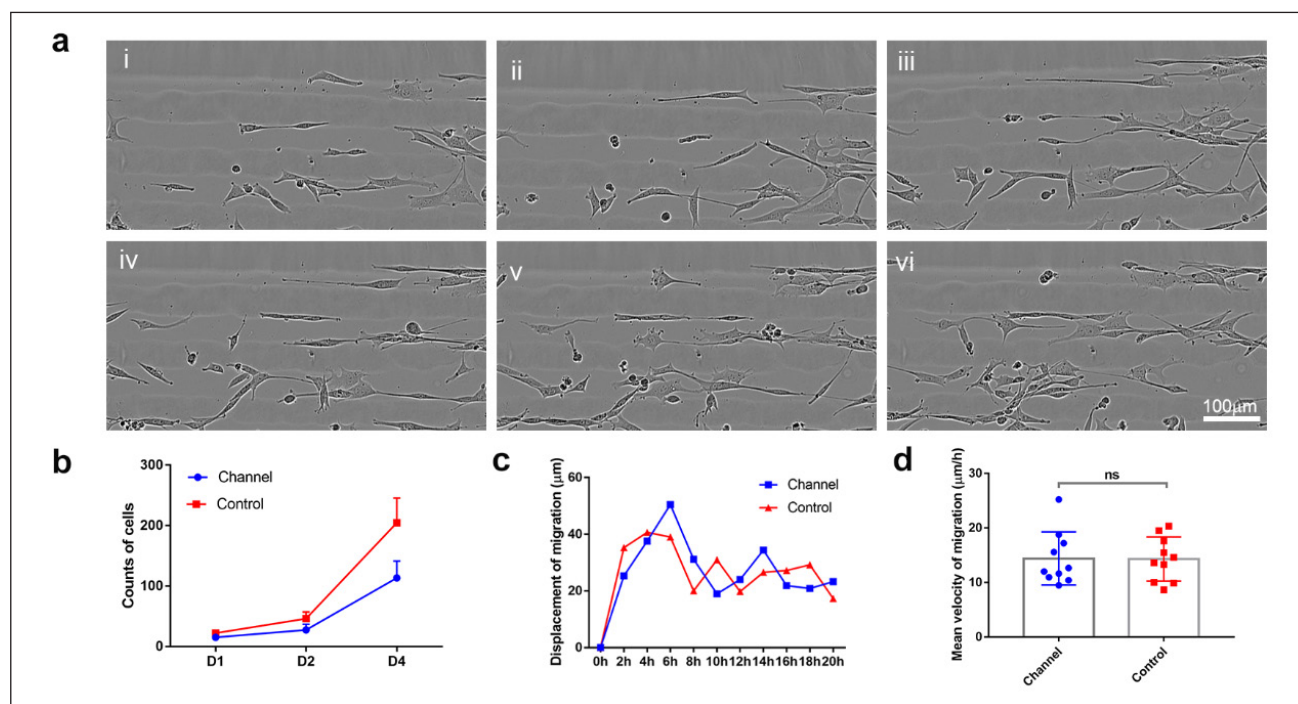


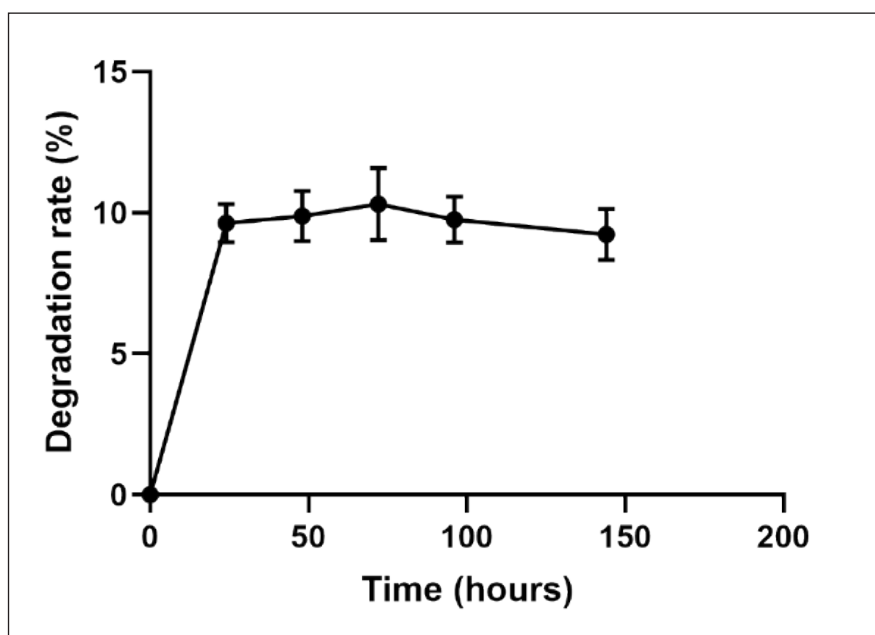
Figure S1. The Young's modulus and concentration of GelMA/PEGDA hydrogel. \*\*\* $p < 0.05$ .



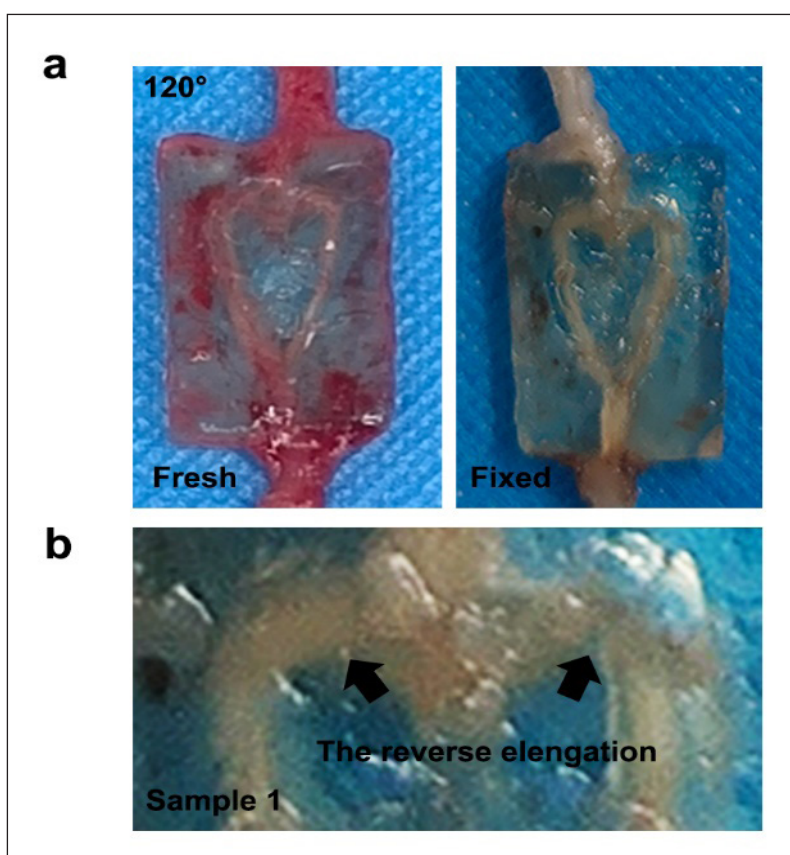
**Figure S2.** The micropatterned scaffold-guided PC12 cell orientation. (a) The schematic illustration of digital light processing (DLP) 3D printing technology. (b) Images of the micropatterned scaffold containing side loops, circular microchannels, and central strip channels. The channel width is 50 μm (scale bar = 800 μm). (c–d) Representative images at different magnifications of PC12 cells in the scaffold after culturing for 3 days. Red indicates rhodamine-phalloidin, and blue indicates DAPI. PC12 cells are oriented and elongated along the direction of microchannels and are interconnected (scale bar = [c] 100 μm; [d] 50 μm). (e) The statistical results (i) of cell orientation angle ( $\theta$ ) in the scaffold and a schematic illustration (ii) of the method to determine the orientation angle. (f) Representative images of PC12 cells at 2, 4, and 6 days after seeding in the scaffold. PC12 cells proliferated rapidly and filled the microchannel at 6 days (scale bar = 200 μm).



**Figure S3.** (a) Cell migration and proliferation in the micropatterned scaffold for 12 h, with each image taken at a 2-h interval time (scale bar = 100  $\mu\text{m}$ ). (b) Cell counts on day 1, day 2, and day 4 under the same objective lens. (c) The migration displacement and (d) velocity of PC12 cells in (Channel) and out (Control) of the scaffold.

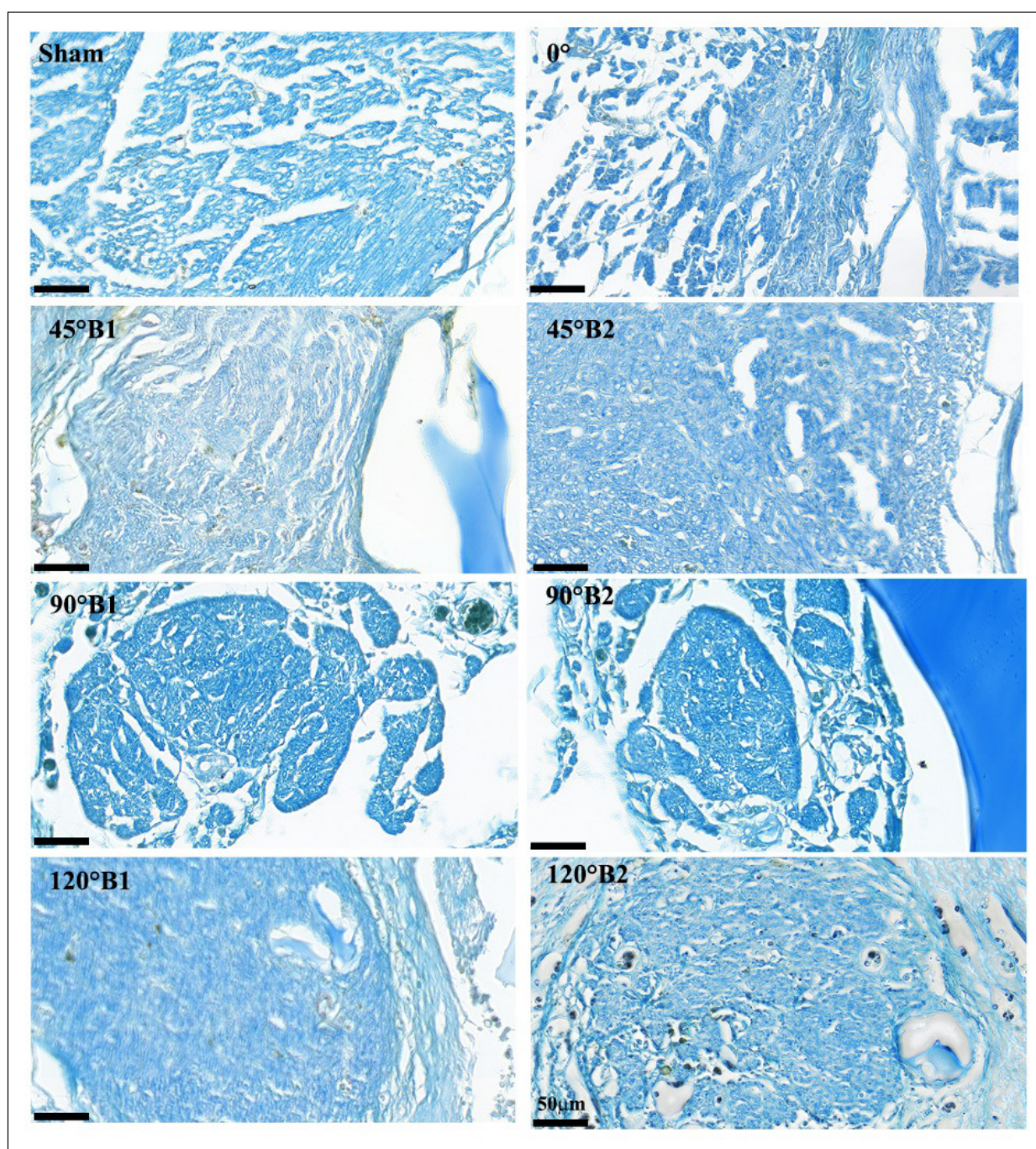


**Figure S4.** Degradation of GelMA/PEGDA hydrogel.

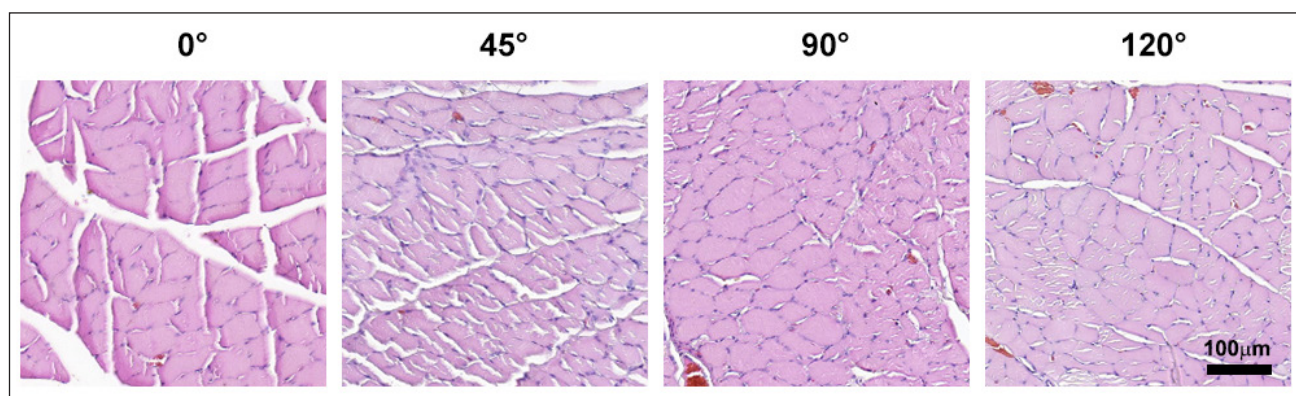


**Figure S5.** The photographs of regenerated nerves in the dual-branched nerve guidance conduits (NGCs) with an angle of 120°. (a) The fresh and fixed nerves in the NGCs. (b) High-magnification image of the regenerated branched nerves (120°). The black arrows indicate the regenerated nerves with a reverse elongation.





**Figure S6.** Images of luxol fast blue (LFB) staining for regenerated nerve tissues with a high magnification (scale bar = 50 μm).



**Figure S7.** The histological analysis of myofibers in the dual-branched nerve guidance conduit (NGC) groups, involving H&E staining of gastrocnemius muscle cross-sections (scale bar = 100  $\mu$ m).