Supporting Information

Ternary Aligned Nanofibers of RGD Peptide-Displaying M13 Bacteriophage/PLGA/Graphene Oxide for Facilitated Myogenesis

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Supplementary methods

Biocompatibility assay for fabricated nanofiber sheets

The biocompatibility of fabricated nanofiber sheets, including PLGA, GO-PLGA, PLGA/RGD, and GO-PLGA/RGD nanofiber sheets, was examined according to the ISO 10993 standards for evaluating the biocompatibility of medical devices. According to the ISO 10993-5 and 10993-12, we immersed fabricated nanofiber sheets with a surface area/extraction medium ratio of 6 cm^2/mL in culture medium [Dulbecco's modified Eagle's Medium (DMEM, Welgene, Daegu, Korea) supplemented with 10 % fetal bovine serum (Welgene) and a 1 % antibiotic-antimycotic solution (containing 10,000 units penicillin, 25 μg amphotericin B and 10 mg streptomycin per mL, Sigma-Aldrich Co., St Louis, MO, USA)] at 37 °C in a humidified atmosphere containing 5% CO_2 for 72 h to obtain sample extracts. The C2C12 skeletal myoblasts were seeded at a density of 5×10^4 cells/mL into 96-well plates, and incubated for 24 h. Thereafter, the culture medium was replaced by the set of sample extract serial dilutions, and further incubated for 24 h. After then, the cells were incubated with cell counting kit-8 (CCK-8, Dojindo, Kumamoto, Japan) solution for another 2 h in the dark at 37 ºC. The absorbance values were determined by using SpectraMax® 340 plate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. The relative cell viability was determined as the percentage of the optical density value in the cells to the optical density value of control groups. The control groups involved DMEM supplemented with 10 % fetal bovine serum and a 1 % antibiotic-antimycotic solution.

Quatification of myoblast alignment ratio

To quantify the myoblast alignment ratio, the immunofluorescence images of C2C12 skeletal myoblasts on random and aligned nanofiber sheets were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). For myoblast orientation, the angle of 0° corresponded to parallel alignment from the direction of aligned nanofibers and the main axis of myoblasts, and the absolute angle of 90° denoted perpendicular alignment.

Supplementary figures

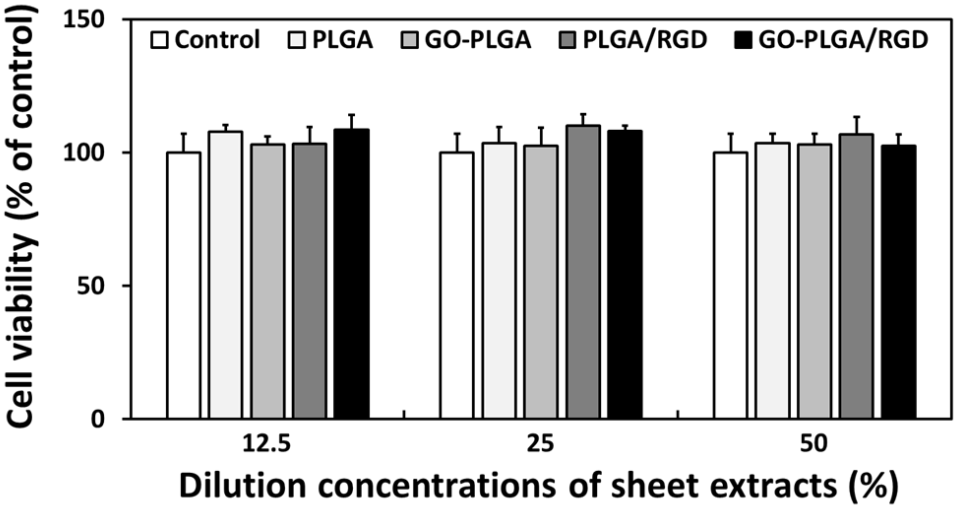

Figure S1. Cell viability of C2C12 skeletal myoblasts after cultured in extraction medium for 24 h. The control groups involved DMEM supplemented with 10 % fetal bovine serum and a 1 % antibiotic-antimycotic solution. The data are presented as the average ± SD of at least three independent experiments, each performed in duplicate on different samples.

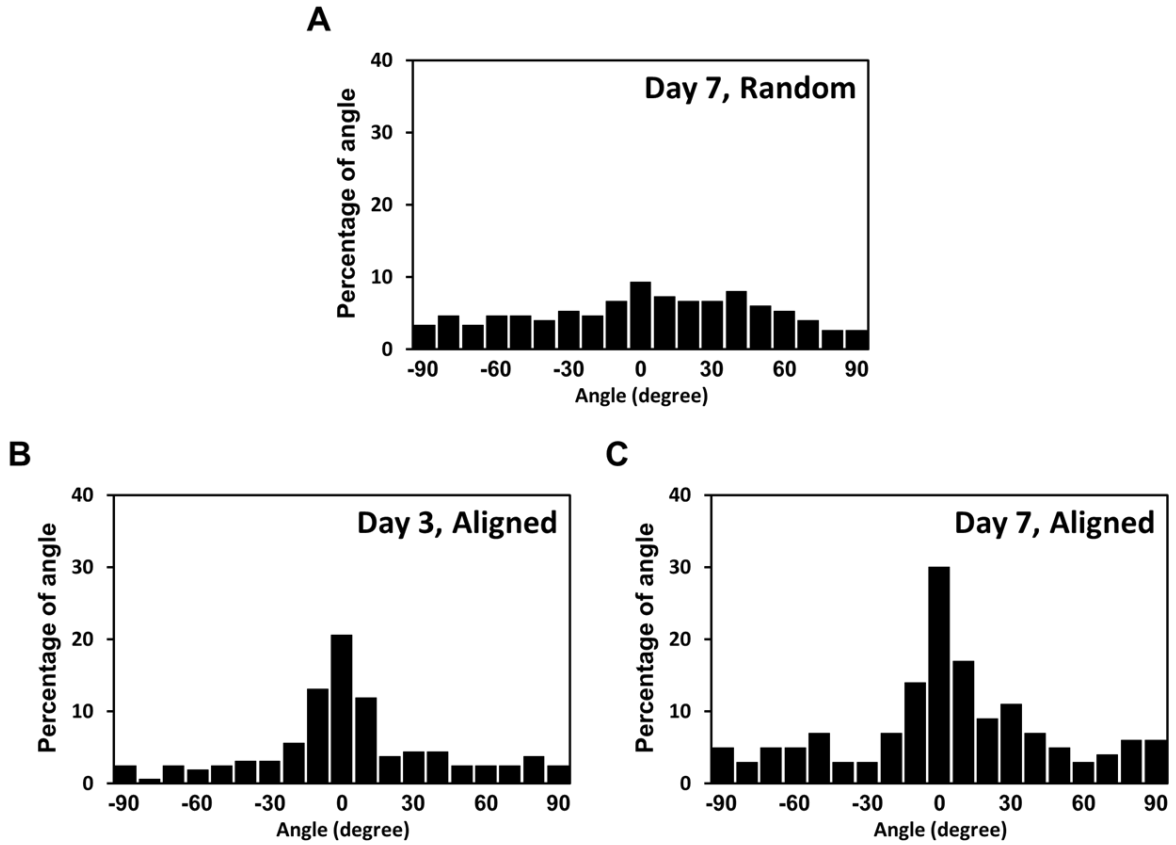

Figure S2. Normalized histograms of myoblast alignment on **(A)** random GO-PLGA/RGD nanofiber sheets at day 7, **(B)** aligned GO-PLGA/RGD nanofiber sheets at day 3 and **(C)** aligned GO-PLGA/RGD nanofiber sheets at day 7. The alignment of myoblasts was quantified by determining the angles between the direction of aligned nanofibers and the main axis of myoblasts.