Engineered Whole Cut Meats Assembled of Cell Fibers Constructed by Tendon-Gel Integrated Bioprinting

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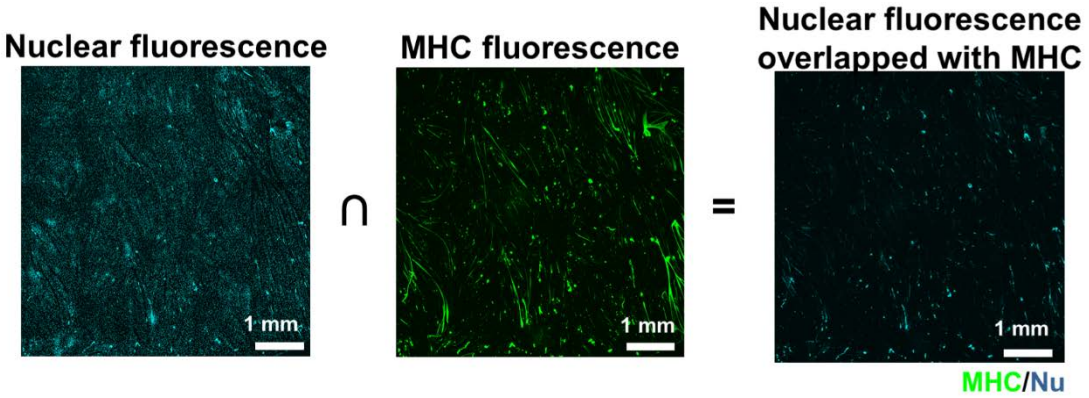
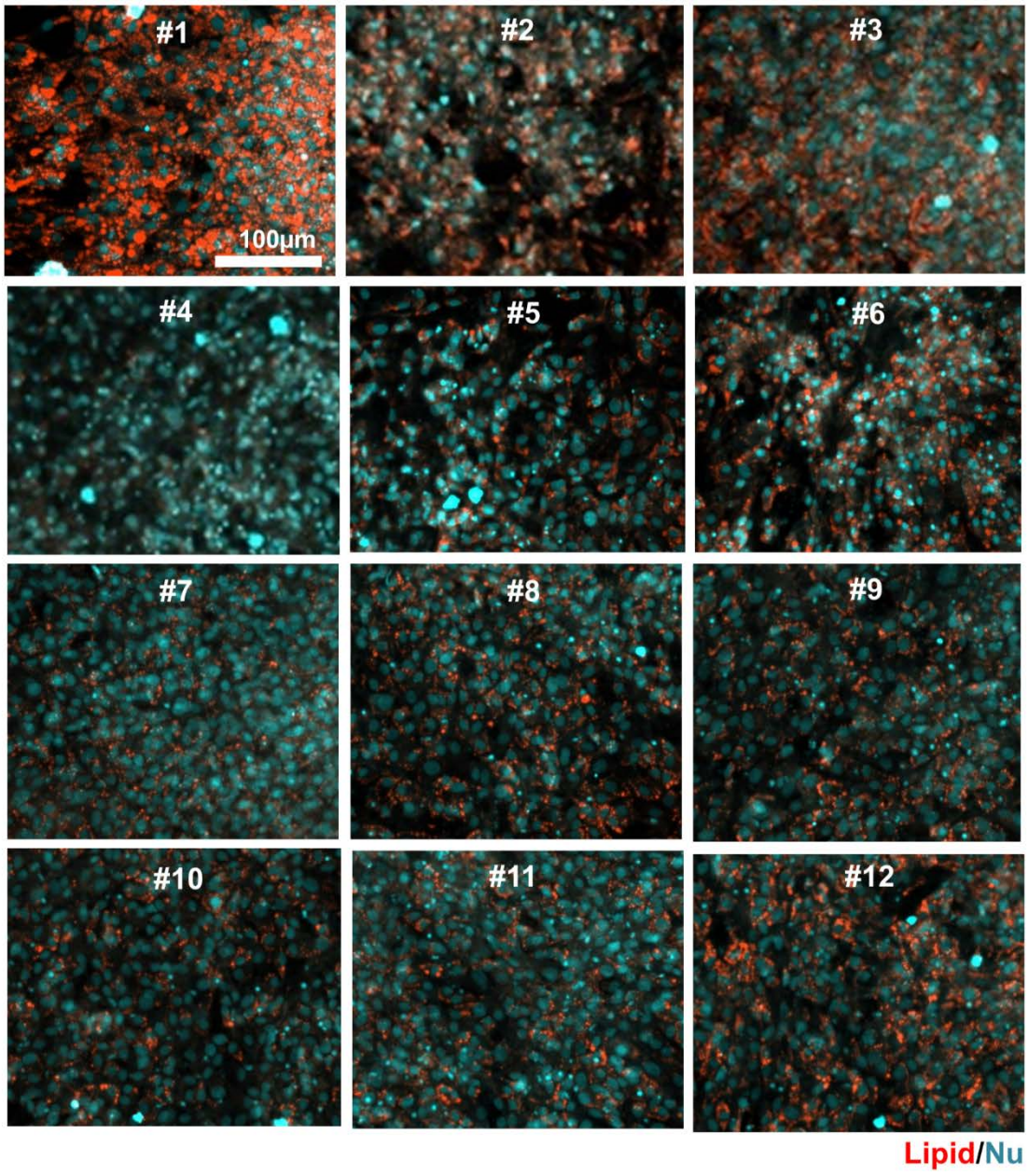
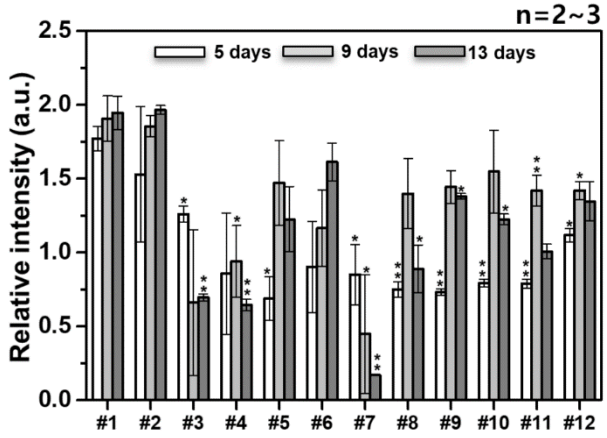


Supplementary Fig. 1 Measurement method of differentiation ratio of bSCs into muscle cells. 'Nuclear fluorescence' is divided by 'Nuclear fluorescence overlapped with MHC'. Nuclear fluorescence overlapped with MHC is obtained in ImageJ software. (green: MHC & blue: nucleus)

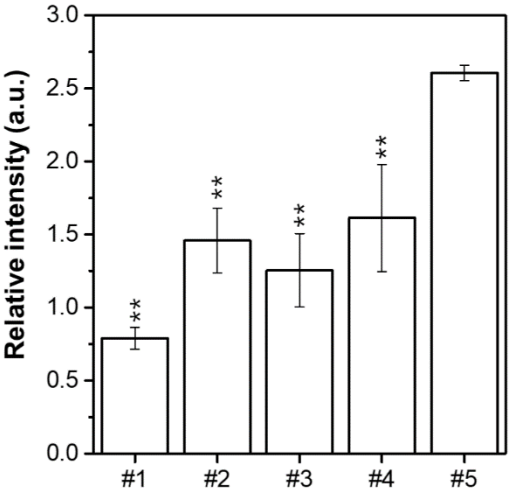


Supplementary Fig. 2 Fluorescence images of adipogenesis of bADSCs by 12 combinations of free fatty acids in DMEM. The used bADSCs were extracted from a subcutaneous fat and all images were taken on day 5. (red: lipid & blue: nucleus)



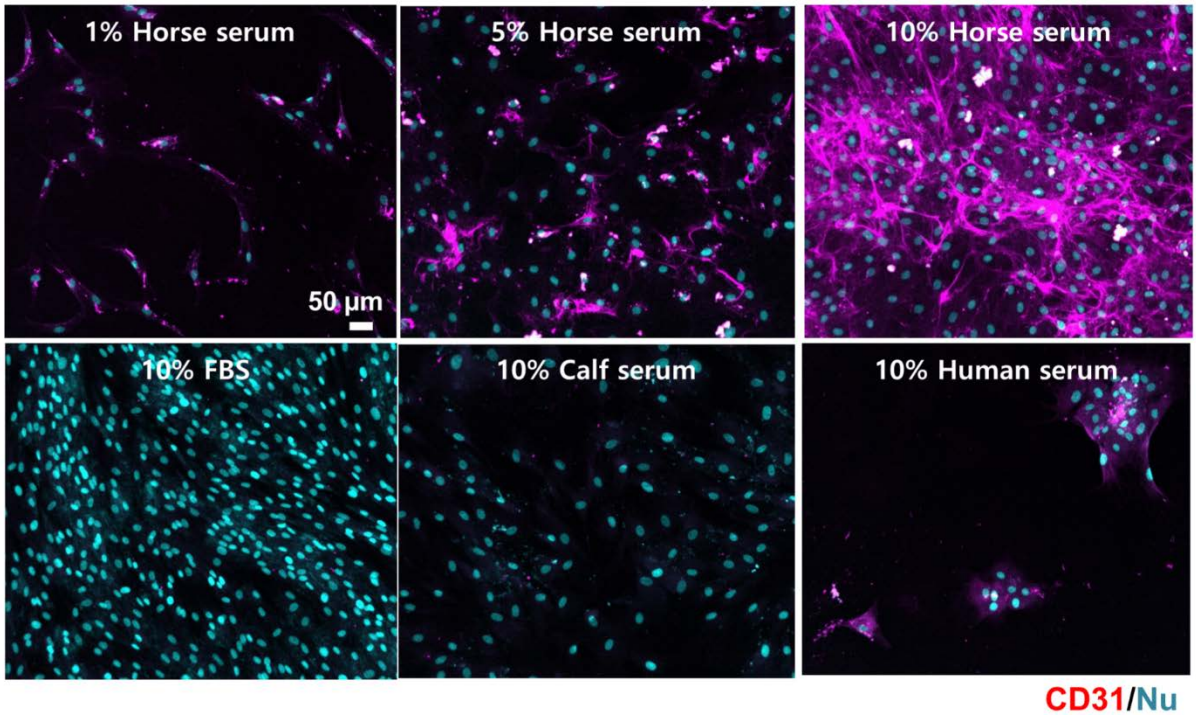
#1	the 7 free fatty acids mixture
#2	Pristanic acid + phytanic acid
#3	Erucic acid + elaidic acid + oleic acid + palmitoleic acid + myristoleic acid
#4	Rosiglitazone
#5	Pristanic acid
#6	Phytanic acid
#7	Erucic acid
#8	Elaidic acid
#9	Oleic acid
#10	Palmitoleic acid
#11	Myristoleic acid
#12	Oleic acid + myristoleic acid + palmitoleic acid

Supplementary Fig. 3 Adipogenesis of bADSCs from a kidney fat tissue. Adipogensis ratio (left) of 3D gel drop cultured bADSCs derived by 12 combinations of free fatty acids (right) in DMEM on day 3, 9, and 13. Statistical significance was calculated with 2-ways ANOVA with Tukey multiple comparison test, n=12 drops tissues/per condition and 2 pictures per drop. *P<0.05, **P<0.01; error bars represent mean ± s.d.

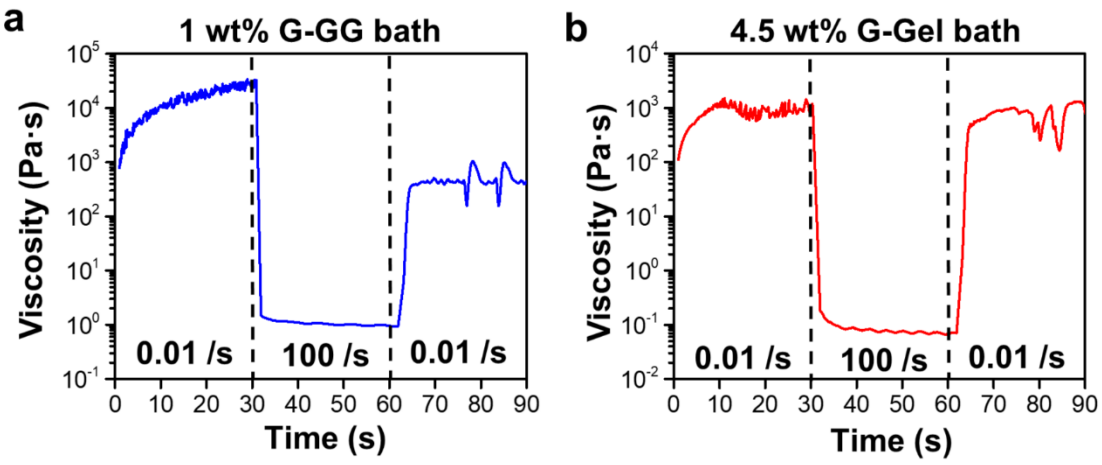


#1	DMEM + insulin 10μg/mL
#2	DMEM + insulin 10μg/mL + troglitazone 40μM
#3	Bovine endothelial medium + insulin 10μg/mL
#4	Bovine endothelial medium + insulin 10μg/mL + troglitazone 40μM
#5	DMEM + the 7 FFA

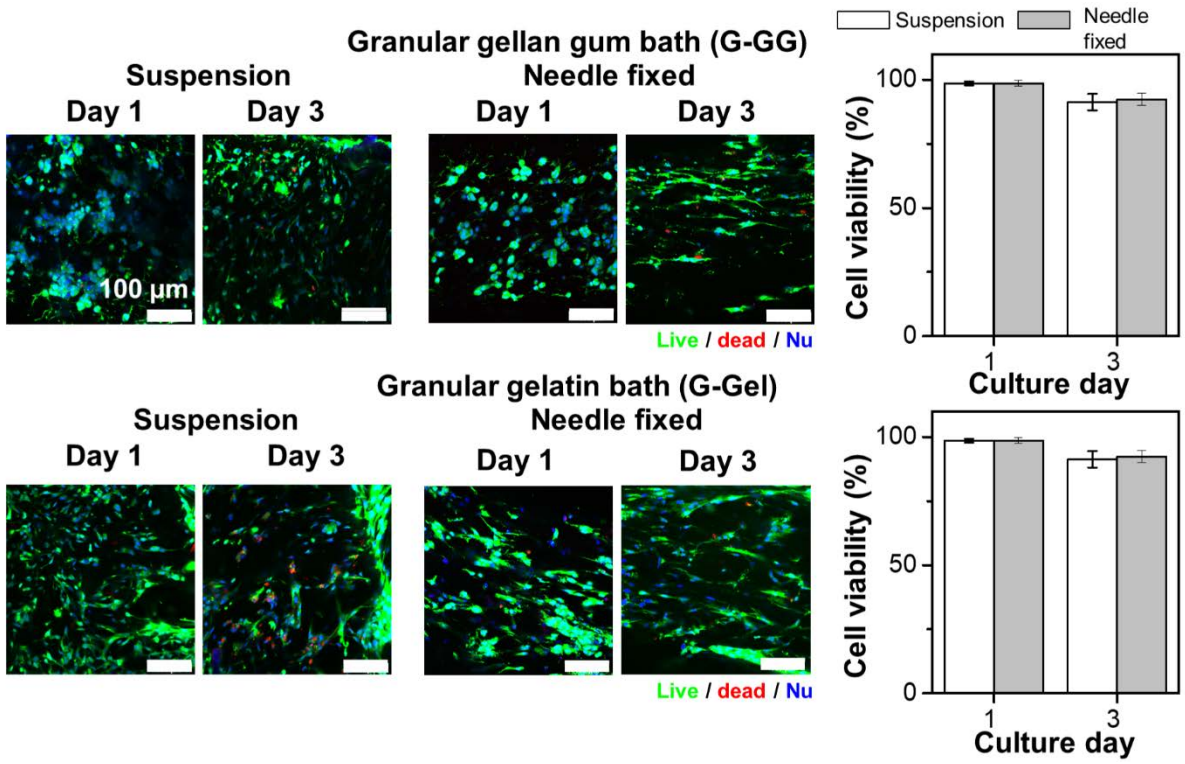
Supplementary Fig. 4 Comparison of free fatty acids and conventional adipogenesis conditions. Adipogensis ratio (left) of 3D gel drop cultured bADSCs derived by several conditions (right) on day 7. The used bADSCs were extracted from a subcutaneous fat. 1-way ANOVA with Tukey multiple comparison test, n=3 drops tissues/per condition. **P<0.01; error bars represents mean ± s.d.



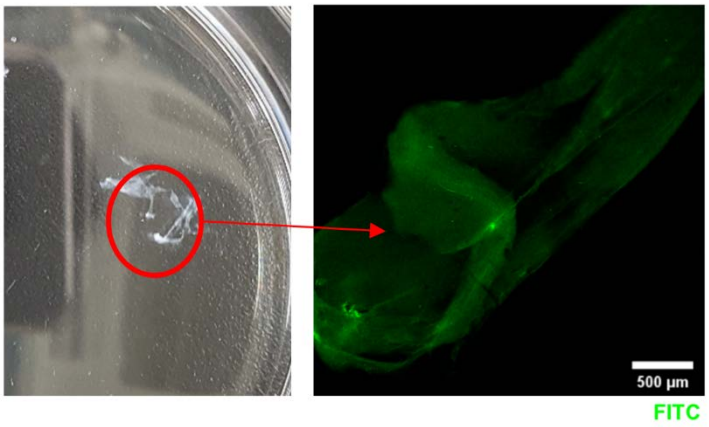
Supplementary Fig.5 Fluorescence images of vasculogenesis of bADSCs depending on serum conditions. The used bADSCs were extracted from a subcutaneous fat tissue and cells were stained with CD31 (red) and nucleus (blue) on day 7.



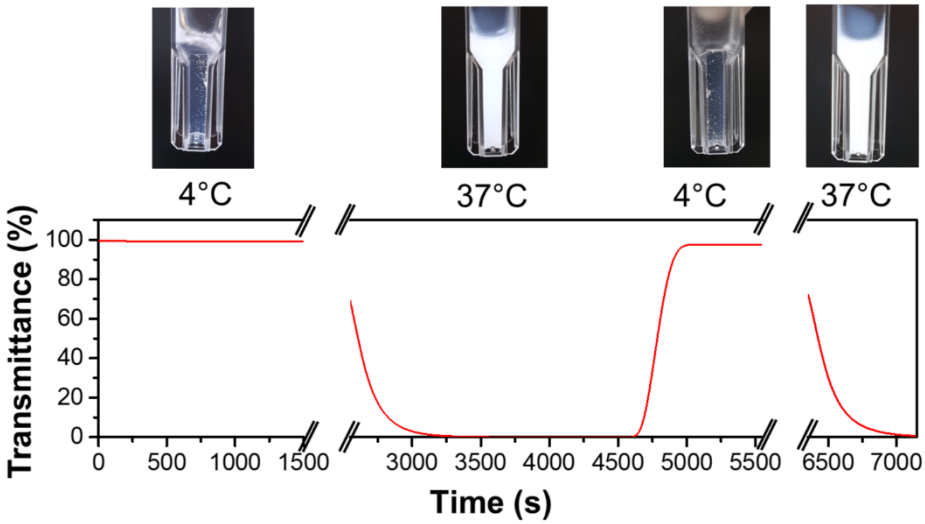
Supplementary Fig. 6 Rheological measurement of G-GG & G-Gel. Viscosity was measured in 3 steps of controlled shear rate mode; low-shear rate (0.01 /s) for 30 s, high-shear rate (100 /s) for 30 s, and low-shear rate (0.01 /s) for 30s.



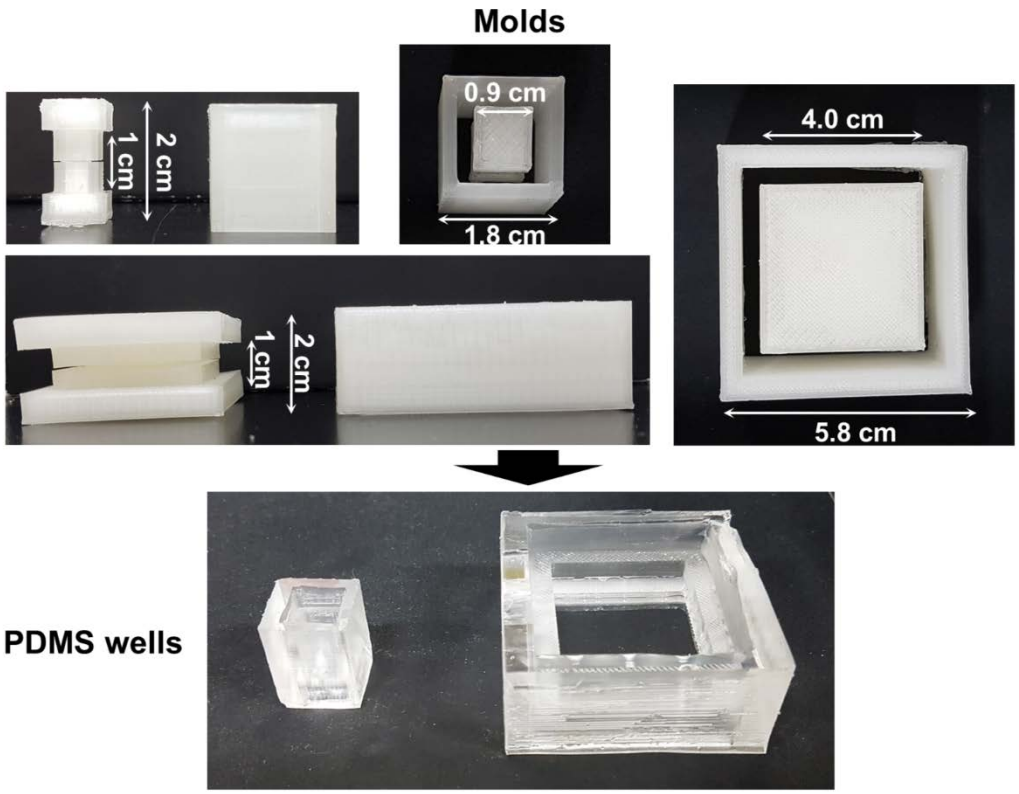
Supplementary Fig. 7 Cell viability of bSCs printed inside G-GG and G-Gel. Error bars represents mean ± s.d. (green: live cells, red: dead cells, and blue: nucleus)



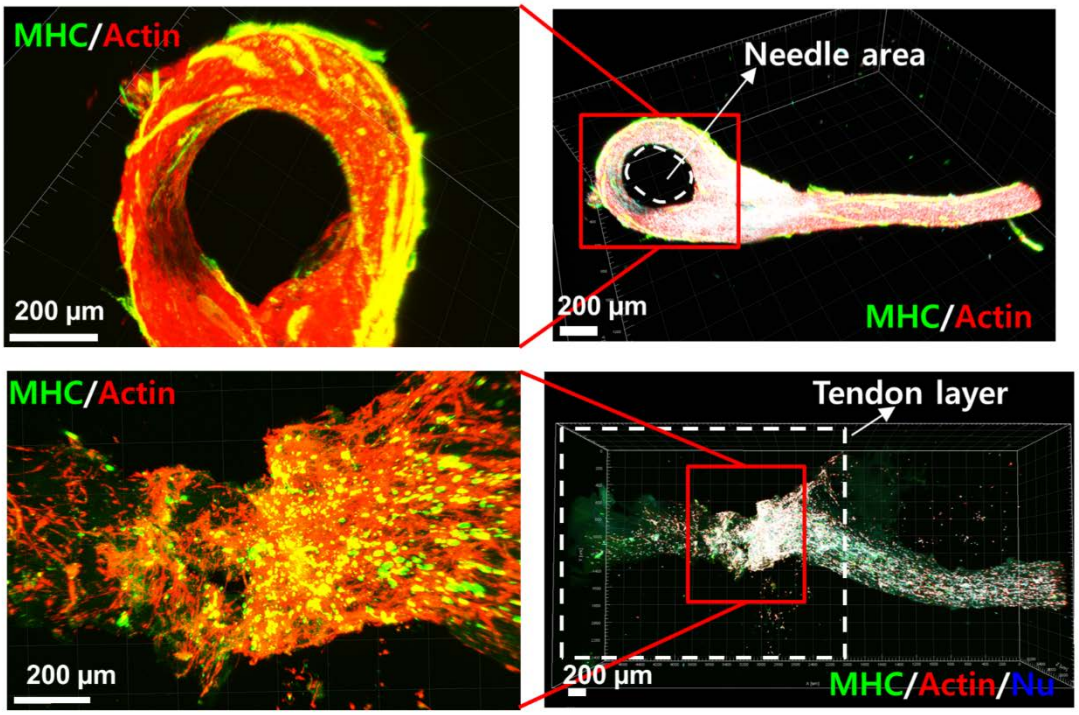
Supplementary Fig. 8 Remaining G-GG in printed structure. The optical image & fluorescence image of fibrin gel printed inside FITC-conjugated G-GG bath after the removal process that it was immersed at 50 mM Tris-HCl buffer (pH 7.4) for 4h. The buffer was replaced with a fresh one every 1h.



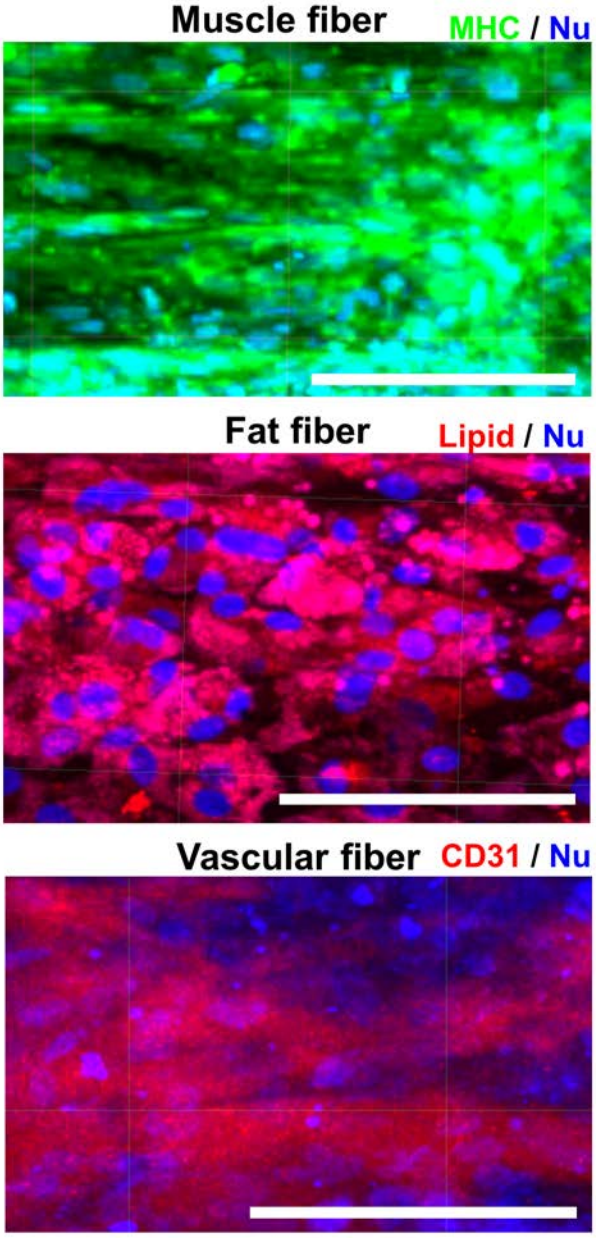
Supplementary Fig. 9 Sol-gel behavior of collagen nanofiber solution (CNFs). The optical images (upper) and transmittance (lower) of 4 wt% CNF fabricated from the mixture of collagen type I & III at steps of 4℃, 37℃, 4℃, and 37℃.



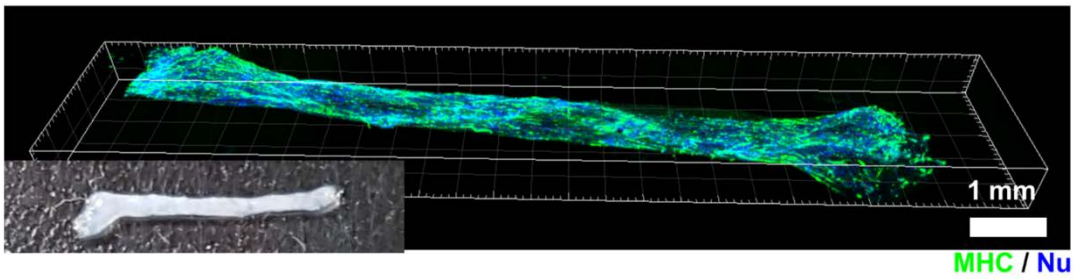
Supplementary Fig. 10 The fabrication of PDMS wells for TIP. The optical images of PDMS wells (lower) through the molds fabricated by FDM 3D printer (upper).



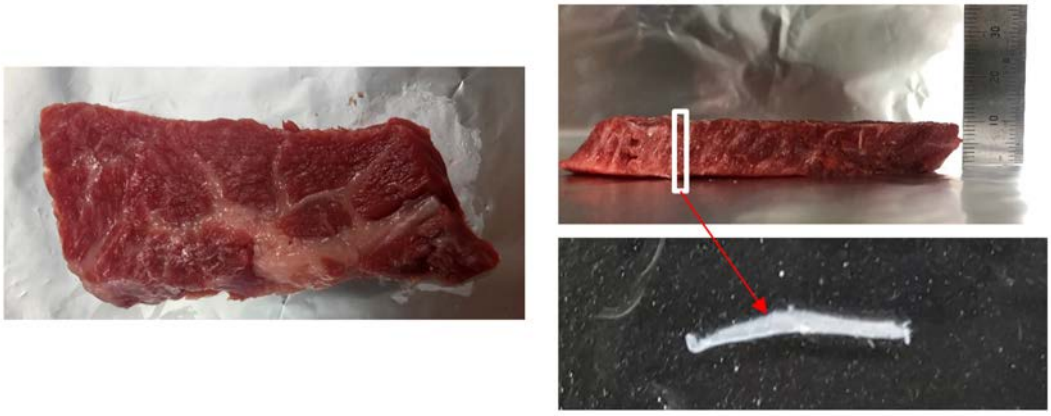
Supplementary Fig. 11 Comparison of connection areas in needle fixed culture and TIP. Fluorescence images (green: MHC, red: actin, and blue: nucleus) of bSCs tissues in needle fixed culture and TIP.



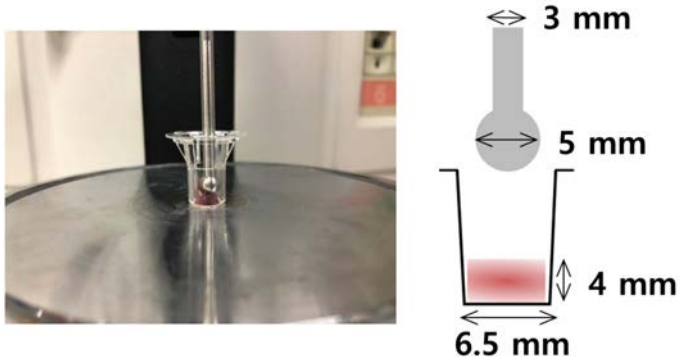
Supplementary Fig. 12 Magnified fluoresce images of muscle, fat, vascular fibers fabricated by TIP. Muscle fiber on day 4 of differentiation (top, green: MHC & blue: nucleus, fat fiber on day 7 of differentiation (middle, red: lipid & blue: nucleus), and vascular fiber on day 7 (bottom, red: CD31 & blue: nucleus). Scale bars, 100 μm.



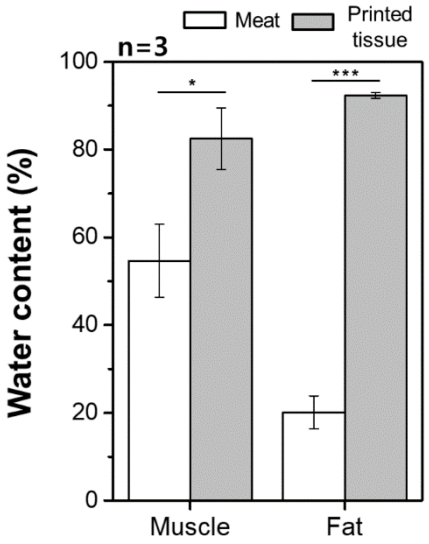
Supplementary Fig. 13 bSCs fiber fabricated by double-sided TIP. Fluorescence (green: MHC & blue: nucleus) and optical (inset) images of bSCs fiber formed by double-sided TIP on 7 day of differentiation. In this double-sided TIP, first printing was conducted same as general TIP and second printing was conducted at close to first printing area after flipping of the PDMS well. The two cell fibers fused into one fiber during culture.



Supplementary Fig. 14 Optical images of commercial meat. The color of the collected fibrous tissue from commercial meat was decolorized during 4% paraformaldehyde fixation.



Supplementary Fig. 15 Compressive modulus measurement set-up. The measurement was conducted after the pressing the cell fibers and fibrous tissues in the cup.



Supplementary Fig. 16 Water content measurement of muscle and fat cell fiber from commercial meat and TIP-derived. The weight measurement was conducted before and after freeze drying of fixed cell fibers. Statistical significance was calculated with 1-way ANOVA with Tukey multiple comparison tests. *P<0.05 and ***P<0.001; error bars represent mean ± s.d.

Supplementary Movie 1. SBP of bSCs inside G-GG

Supplementary Movie 2. SBP of bSCs inside G-Gel

Supplementary Movie 3. TIP of bSCs

Supplementary Movie 4. 3D image of the TIP-derived bSCs tissue on 3 day of differentiation (green: MHC, red: actin, and blue: nucleus)

Supplementary Movie 5. Multiple tissue fabrication by TIP

Supplementary Movie 6. Whole 3D image of the muscle fiber by TIP on 4 day of differentiation. (green: MHC & blue: nucleus)

Supplementary Movie 7. Whole 3D image of the fat tissue by TIP on day 7. (red: lipid & blue: nucleus)

Supplementary Movie 8. Whole 3D image of the vascular tissue by TIP on day 7 of differentiation. (red: CD31 & blue: nucleus)