Biofabrication of 3D-printed fibrous scaffolds for large muscle tissue engineering: enhancing scalability, myotube formation and surgical handling

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Supporting Information

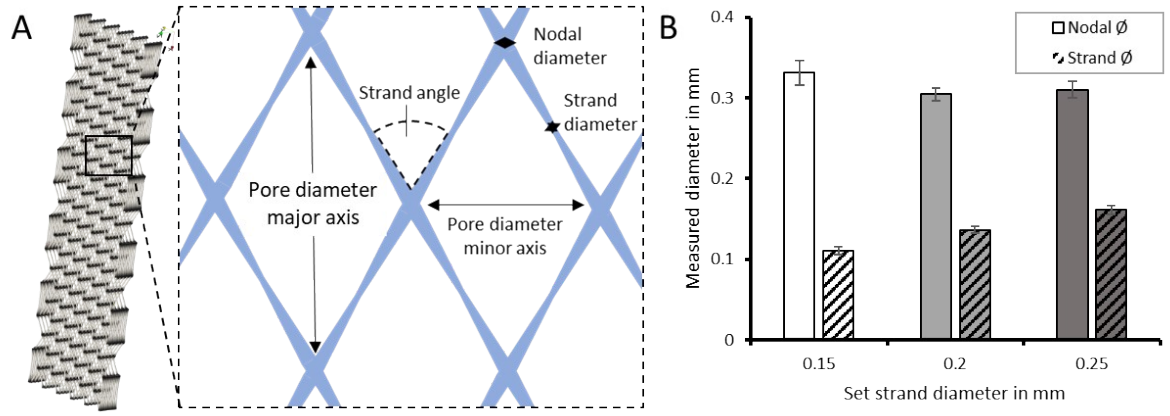


Figure S1 (A) Scaffold design parameters and (B) analysis of the measured diameter compared to the set strand diameter.

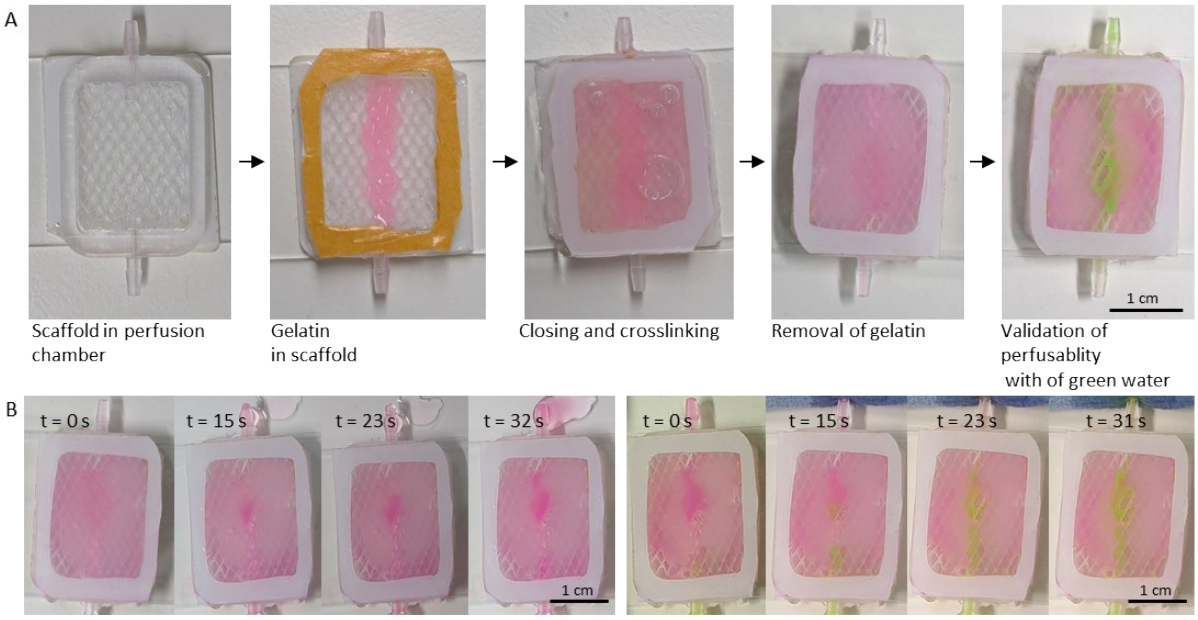


Figure S2 (A) Fabrication of a perfusable sacrificial gelatin channel in the collagen-matrigel matrix in the printed scaffold. (B) Removal of the gelatin channel by perfusion of pink water at 37°C, 50 µl/min, and visualization of the perfusion with green water at 37°C, 50 µl/min.

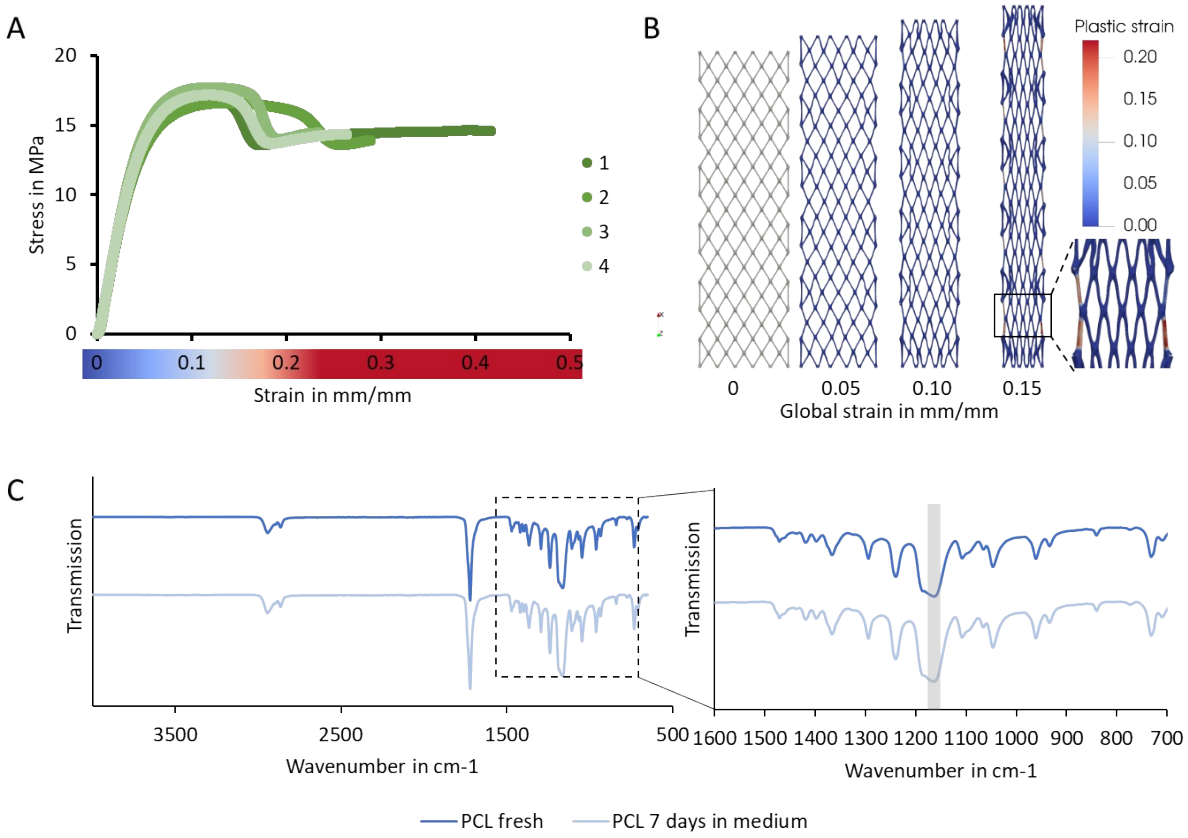


Figure S3. (A) Tensile testing of PCL bulk material (n=4) and (B) simulation of local plastic deformation with applied global strain of up to 15% strain. (C) FTIR analysis of PCL before and after 7 days incubation in growth medium at 37°C.

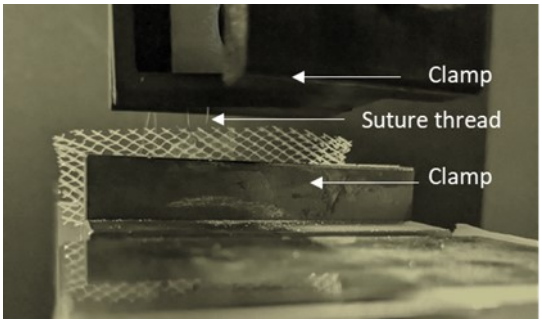


Figure S4. Experimental setup of the suture retention test utilized to measure the maximal retention force.

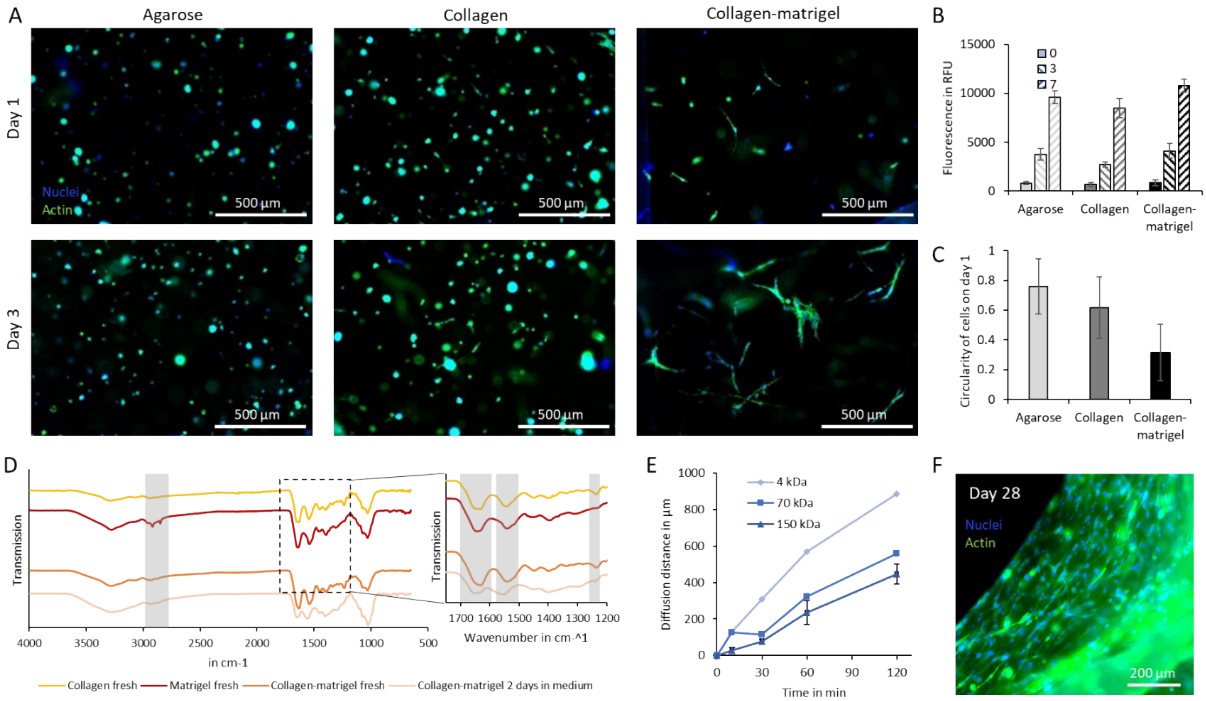


Figure S5. C2C12 in growth media in 0.5 mg/ml agarose, 0.3 mg/ml collagen and 0.3 mg/ml collagen-0.17 ml/ml matrigel. (A) Microscopic images of the cells on day 1 and day 3 after seeding in the gel (nuclei: blue, actin: green) and (B) metabolic activity measured with a cell titer blue assay on day 0, 3 and 7 after seeding (n = 3). (C) Quantification of cell morphology on day 1 with a perfectly round cell assigned to the value 1 (n=3, number of cells analyzed per group n>134). (D) Diffusion of dextran of different sizes in collagen-matrigel. (E) FTIR spectra of bioink components and their mixture before and after 2 day incubation in growth media at 37 °C. (F) Microscopy of the cells in collagen-matrigel in the scaffold after 28 days in differentiation media (nuclei: blue, actin: green).

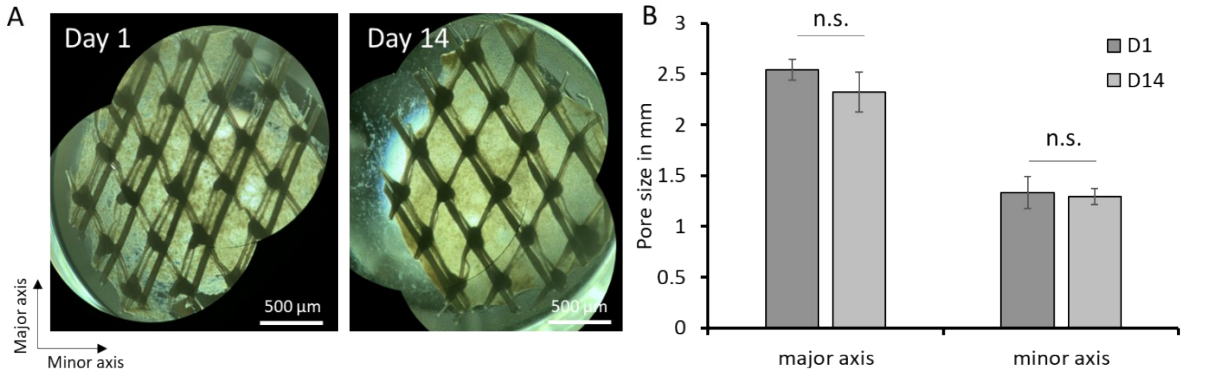


Figure S6. (A) Microscopic images of C2C12 differentiation over the course of 14 days in collagen-matrigel in the scaffolds and (B) quantification of scaffold pore sizes (n = 5, statistical significance for p <0.05 with n.s. for no significance).*

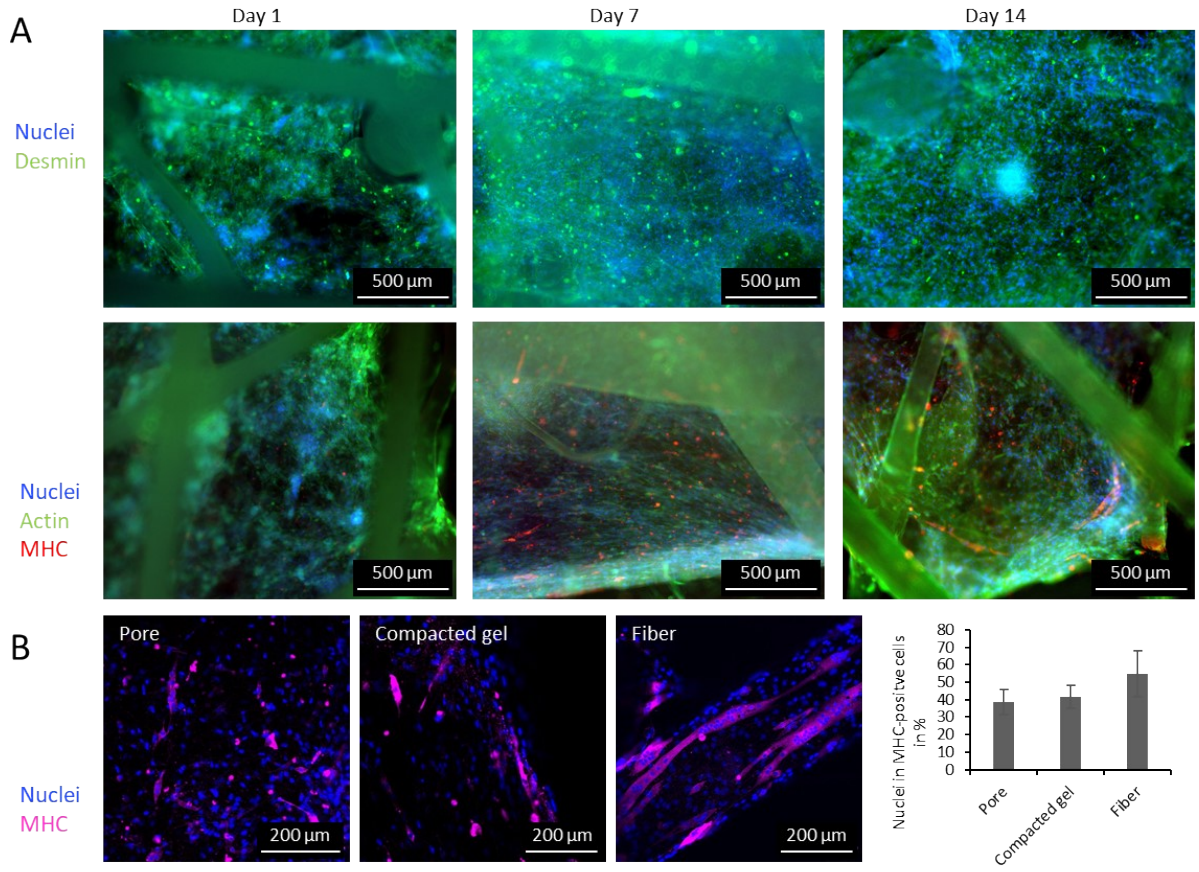
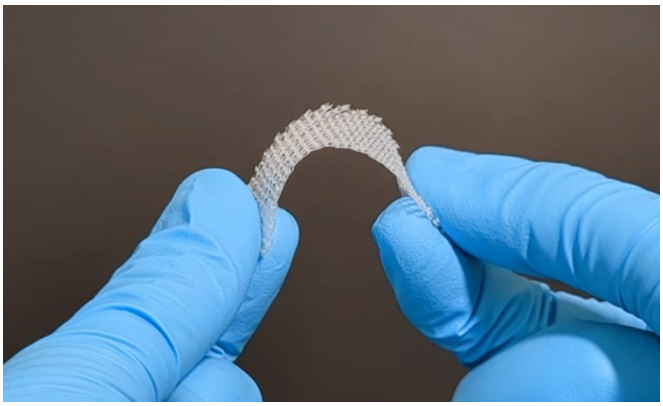
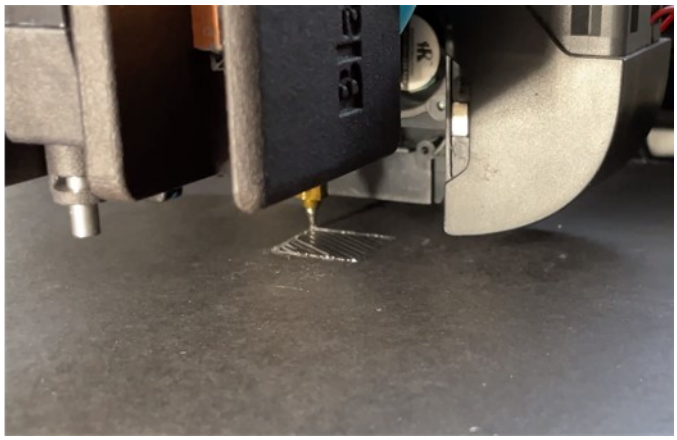


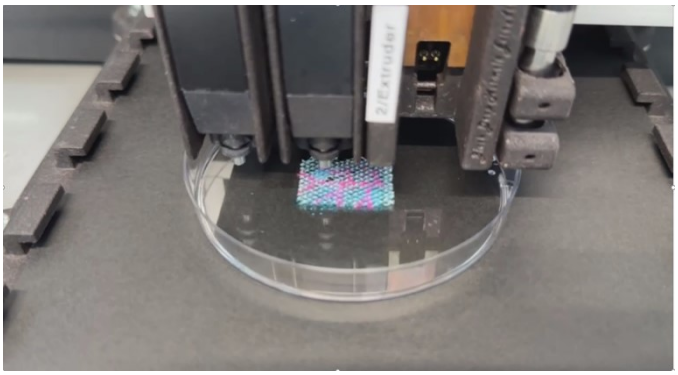
Figure S7. 3D cell culture of C2C12 myoblasts in a 0.3 mg/ml collagen-0.17 mg/mg matrigel hydrogel in scaffolds and differentiation into myotubes. (A) Fluoresence microscopy of samples cultured in differntiation media for 1, 7 and 14 days and staining for early differentiation marker desmin (nuclei: blue, desmin: green) and staining for later stage differentiation marker myosin heavy chain 4 (MHC)(nuciei: blue, actin: green, myosin heavy chain-4: red). (B) Fluoresence microscopy of day 14 of differentiation in different areas of a cell seeded scaffold(nuciei: blue, myosin heavy chain-4: magenta) and quantification of percentage of nuclei in MHC positve cells (n = 3, N=1, statistical significance for p <0.05).*



Video S1. Handling of scaffolds.



Video S2. Visualization of the FFF-spinning and subsequent drop on demand bioprinting process.



Video S3. Visualization of the drop on demand bioprinting into the scaffold using multiple bioinks.