Angiogenesis-Myogenesis Coupling in an In-Bath Bioprinted Skeletal Muscle Tissue provides a Regeneration-competent Satellite Cell Niche

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1. Introduction

Skeletal muscle-resident satellite cells (SC), which possess intrinsic self-repair capacity upon regenerative stimuli such as tissue injury. However, limited long-term maintenance (e. g. loss of stem cell potential during culture) of SCs function hampers their therapeutic efficacy for the treatment of Volumetric Muscle Loss (VML) or Duchenne Muscular Dystrophy (DMD). Biofabrication techniques such as 3D bioprinting, electrospinning, and molding have enabled the recapitulation of mature myofibers and vascular networks. SC-vasculature or SC-myofiber cellular crosstalk are a critical factor for retaining the stem cell potential. Therefore, we suggest a 3D bioprinting-based direct endothelial cell patterning method which leads to a spontaneous vasculature formation within mature MYOG-positive myofibers. Angiogenesis-myogenesis coupling has been confirmed by marker gene expressions and has been maintained during 14 days of culture. In addition, secretion of myofiber-derived quiescence-inducing niche factor Oncostatin M increased steadily until 7 days of culture. Moreover, pro-regenerative cytokines including IGF-1 and SDF-1 was upregulated upon vascularization, while pro-inflammatory cytokines known to induce apoptotic death of myoblasts were downregulated.

2. Materials & Methods

We first prepared skeletal muscle decellularized extracellular matrix (mdECM) bioink following the procedure depicted in the schematic below. Then we directly printed human umbilical vein endothelial cells (HUVEC)-laden mdECM bioink as patterns, into human skeletal muscle myoblast-laden mdECM bioink which behaved as a supporting bath. First, spatial division of myoblast and endothelial cells after patterning were confirmed by immunofluorescence staining. Then we conducted qRT-PCR to compare the myogenesis- and angiogenesis-related gene expression level of patterned and non-patterned group (randomly mixed). Second, we confirmed myogenesis via immunofluorescence staining against F-actin and Myogenin (MYOG), a key transcription factor for myogenesis to proceed. Third, we confirmed that higher EC proportion leads to enhanced expression of marker genes which regulates angiogenesis-myogenesis coupling. Expression of late myogenesis genes implied this coupling was maintained for long-term via optimizing the pattern-pattern distance. Lastly, we conducted cytokine array to compare the different expression profile of pro-inflammatory and pro-regenerative cytokines between non-vascularized and vascularized tissues.

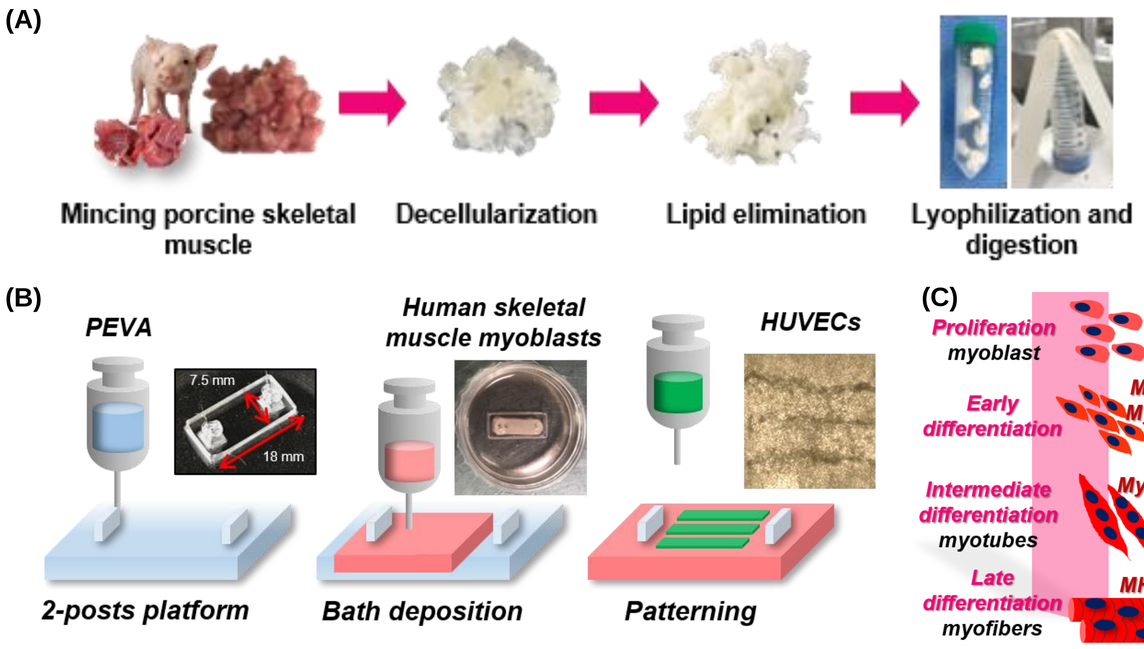

Figure 1. (A) Schematic of mdECM bioink preparation process. (B) In-bath printing method. (C) Schematic of different stages of myogenesis.

3. Results

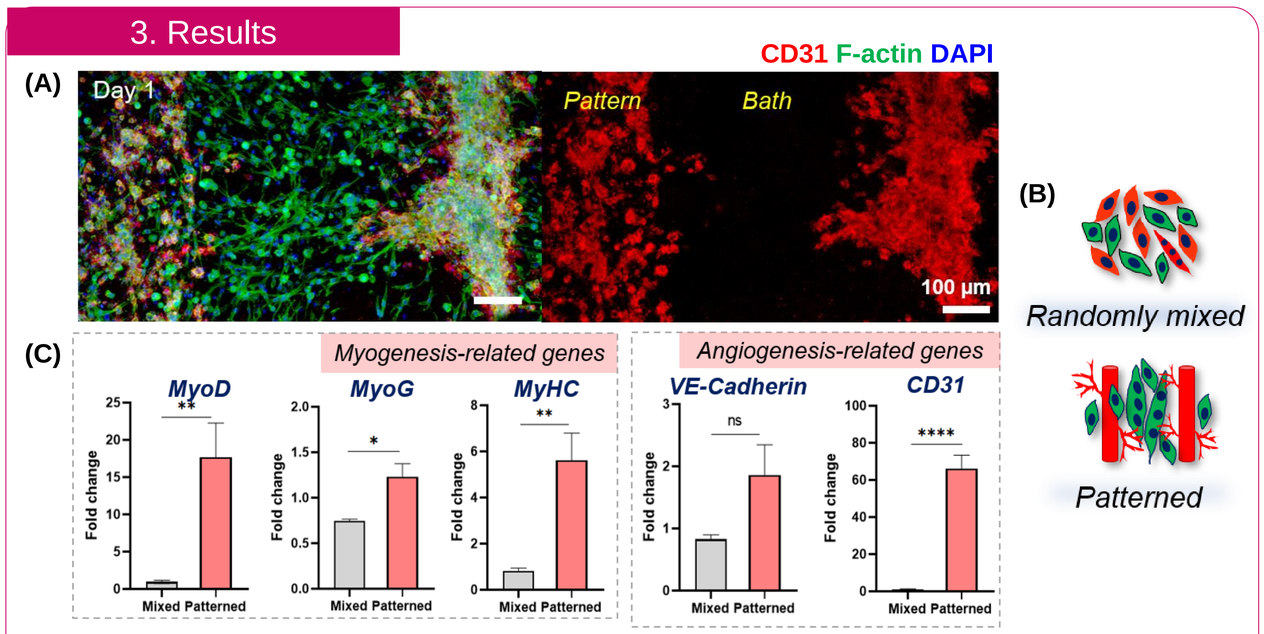

Figure 2. (A) Patterns retained initially printed shapes after in-bath printing (time point: day 1; CD31: endothelial cell marker. F-actin: cytoskeleton marker.) (B) Schematic of randomly mixed vs. patterned tissue. (C) Myogenic and angiogenic marker gene expression altogether increased in the patterned group (time point: day 7; Unpaired t-test).

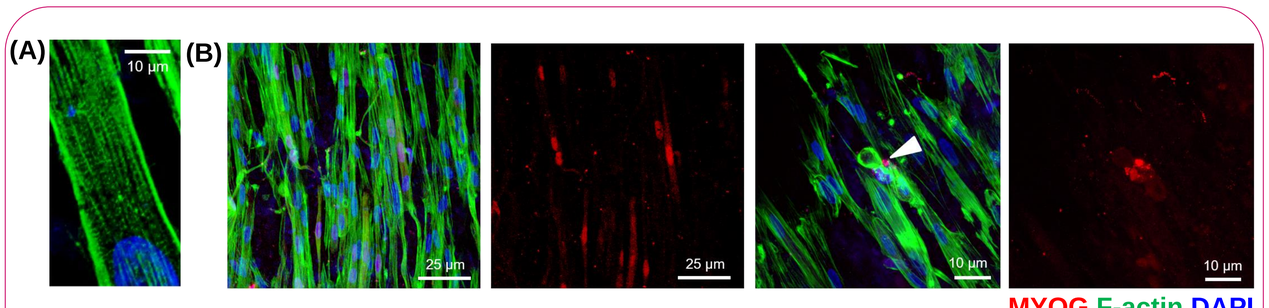

Figure 3. (A) Myofiber striated pattern expression at day 7. (B) MYOG+ multiple nuclei at day 14.

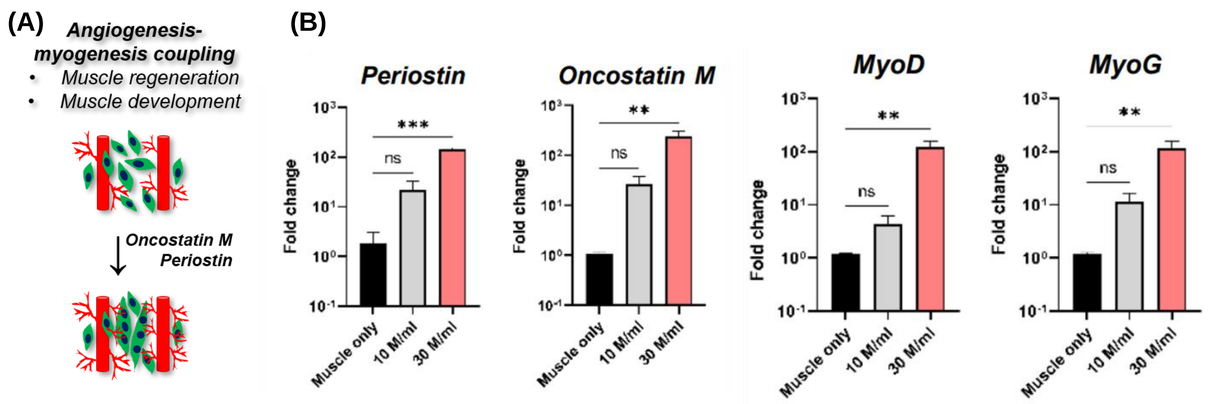

Figure 4. (A) Schematic of angiogenesis-myogenesis coupling. (B) Increased expression of Periostin (muscle ECM component), Oncostatin M (myokine of IL-6 family) and myogenic genes in higher endothelial cell proportion conditions (y axis in log 10 scale; One-way ANOVA test).

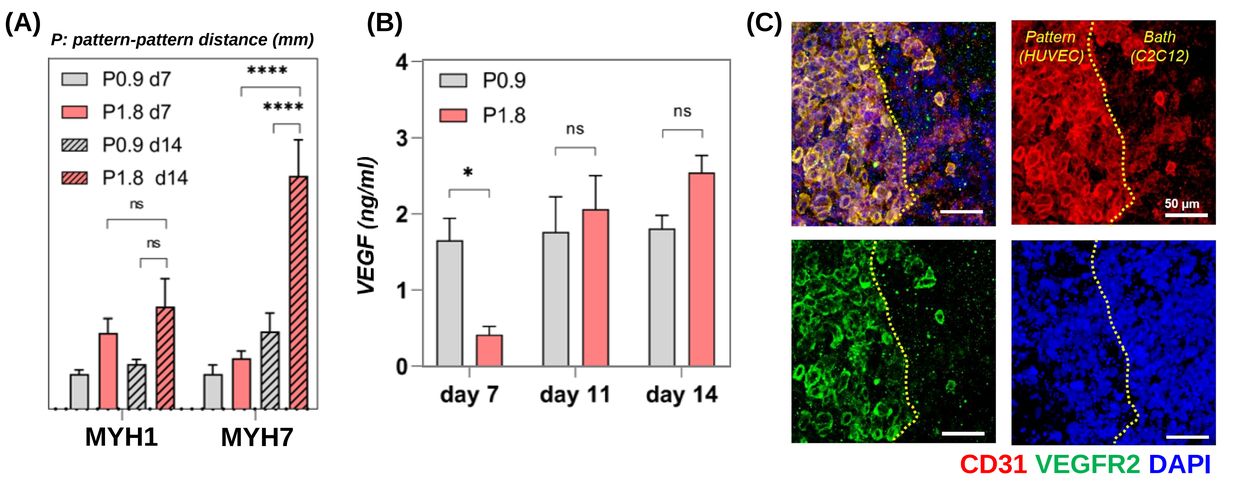

Figure 5. (A) Effect of longer pitch on enhanced muscle maturation. (B) Robust increase of muscle-secreted vascular endothelial growth factor (VEGF) until day 14 (A, B: Two-way ANOVA test). (C) VEGF receptor 2 (VEGFR2) exclusively expressed on HUVECs-laden pattern.

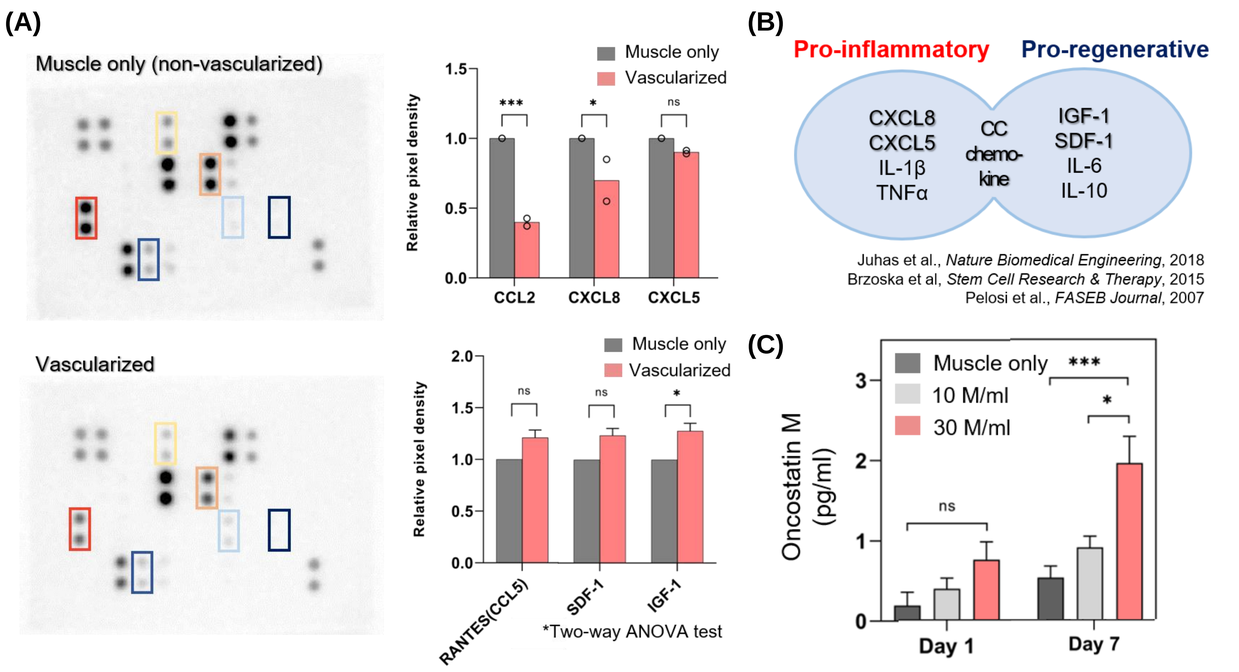

Figure 6. (A) Down0-regulated pro-inflammatory cytokines and up-regulated pro-regenerative cytokines. Time point: day 7. (B) Representative pro-inflammatory or pro-regenerative cytokines. (C) Quantification of niche factor Oncostatin M by ELISA (Two-way ANOVA test).

4. Conclusion

Printed HUVECs and myoblasts stably proliferated at their positions. Enhanced marker gene expression implied that spatial division between different cell types is effective for tissue maturation as well. Robust myogenesis and angiogenesis-myogenesis coupling was confirmed by IF staining (F-actin, MYOG) and marker gene expression including Oncostatin M. Longer pattern-pattern distance resulted in enhanced expression of late myogenesis genes as well as higher amount of muscle-secreted VEGF, confirming it a more suitable condition for long-term maintenance of angiogenesis-myogenesis coupling. Cytokine array confirmed that vascularization provides a pro-regenerative microenvironment which will enhance the survival and stem cell potential of satellite cells.

5. Acknowledgement

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