Biofabrication of Innervated Muscle Tissue for Accelerated Restoration of Muscle Function

Hyun-Wook Kang, In Kap Ko, Cheil Kim, Sang Jin Lee, Anthony Atala, and James J. Yoo
Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine,
Medical Center Boulevard, Winston-Salem, NC 27157, USA

Statement of Purpose: Building a clinically applicable functional muscle tissue with necessary tissue organization and function would be a major advance in reconstructive surgery. To achieve functional recovery *in vivo*, bioengineered muscle tissues must be integrated with the host nervous system following implantation. Failure of innervation results in muscle tissue atrophy. Therefore, timely integration of host nerve into bioengineered muscle tissue is critical to successful recovery of function. To overcome these limitations, we aimed to develop organized muscle tissue constructs with neuromuscular junction (NMJ) *in vitro* and to test innervation efficiency of the muscle construct *in vivo*.

Methods: An integrated organ printing system was developed to print implantable tissue structures using a three-dimensional (3D) CAD model (Figure 1A). Muscle tissue constructs were generated by printing muscle cells (C2C12 cells; 2×10^6 cells/ml) suspended in a mixture of gelatin (40 mg/ml), hyaluronic acid (3 mg/ml), glycerol (10% v/v) and fibrinogen (30 mg/ml), followed by addition of cold thrombin (20~30 U/ml) for 30 min for fibrin gel formation. The bioprinted muscle constructs were cultured to induce differentiation of muscle cells into muscle fibers, and then treated with agrin to pre-pattern acetylcholine receptors (AChR) on the muscle fibers. The bioprinted muscle constructs were integrated with mobilized host sciatic nerve and implanted subcutaneously in nude rats. The constructs were retrieved and examined with histological (H&E) and immunohistological methods (MHC and α-BTX) at the designated time points.

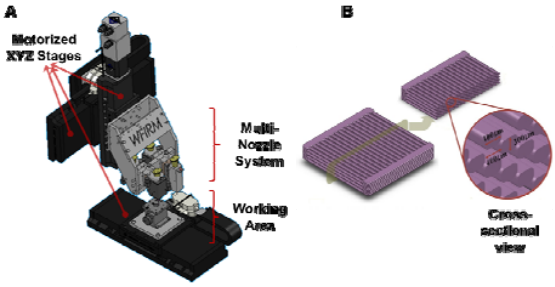

Figure 1. Integrated organ printing system for printing a muscle structure: (A)

Results: The bioprinted muscle tissue constructs maintained their structural organization, consisting of viable muscle fiber bundles in culture (Figure 2). Cell viability was maintained over 80% after printing. At 7 days after differentiation, muscle fibers were formed along the longitudinal direction of the printed muscle construct (Figure 2C).

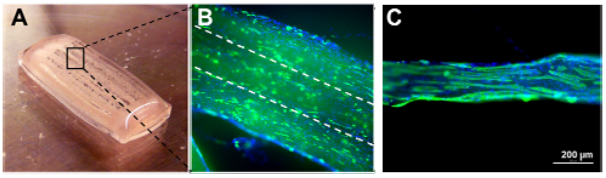

Figure 2. *In vitro* bioprinted muscle construct: (A) gross appearance, (B) live/dead staining at 1 day after printing, and (C) MHC-immunostaining at 7 days after differentiation.

More importantly, the fiber bundles expressed muscle-specific characteristics after induction of cellular differentiation as well as acetylcholine receptor (AChR) expression by agrin treatment. The retrieved muscle constructs showed well-organized muscle fiber structures expressing AChRs and evidence of neural integration with host sciatic nerve (Figure 3).

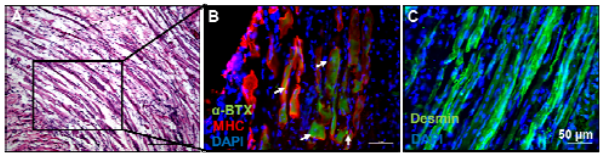

Figure 3. *In vivo* bioprinted muscle construct: (A) H&E staining, (B) MHC/α-BTX-immunostaining, and (C) desmin-immunostaining.

Conclusions: Our findings show that tissue constructs generated with an integrated organ printer are able to maintain structural and functional characteristics of muscle *in vitro* and *in vivo*. The results of this study suggest that creation of innervated volumetric bioengineered muscle using an organ printing system is possible and that muscle function can be recovered in an accelerated fashion.

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References:

[1] Choi JS, Lee SJ, Christ GJ, Atala A, and Yoo JJ, Biomaterials. 2008;29(19):2899-906.
[2] Xu T, Zhao W, Zhu JM, Albanna MZ, Yoo JJ, and Atala A, Biomaterials. 2013;34(1):130-9.