3-D Bioprinting of Skeletal Muscle Tissue Constructs for Accelerated Restoration of Muscle Function

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Introduction

Current management of soft tissue coverage and augmentation involves the use of existing host tissue such as muscle flaps or tissue grafts [1]. In many instances, this approach is not feasible, delaying the rehabilitation process as well as inadequate functional recovery. In this respect, we propose to develop a functional muscle tissue implant using tissue engineering strategies. Recent advances in skeletal muscle tissue engineering present a promising approach to overcome the current limitations involved with structural and functional recovery of injured or diseased muscle tissue [2]. However, the conventional tissue engineering methods are limited by the ability to build volumetric tissue constructs with cellular organization, which are inadequate for replacing extensive muscle defects. In addition to achieve functional recovery of muscle *in vivo*, innervation is critically important following implantation, as failure of innervation leads to muscle tissue atrophy and loss of contractile function [2]. Therefore, timely integration of host nerve into engineered muscle tissue construct is critical to successful recovery of function. In this study we investigated whether 3-D bioprinted muscle constructs could be robust enough to maintain structural and functional characteristics.

Materials and Methods

Human muscle progenitor cells (hMPCs) used for printing skeletal muscle constructs were cultured in high glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. The engineered skeletal muscle constructs composed of multi-layered muscle-like structure were fabricated using integrated organ printing (IOP) system (**Fig. 1A**). The cell viability (live/dead assay) and muscle development of muscle construct (stained with anti-myosin heavy chain (MHC)) were evaluated *in vitro*. Bioprinted muscle constructs were implanted subcutaneously in athymic mice and structural maintenance, skeletal maturation, integration with host tissue (stained by anti-vWF, Neurofilament (NF), and acetylcholine receptors (AChR)) were examined.

Results

The printed skeletal muscle constructs showed 83.7 ± 3.8% of cell viability at 1 day after printing (n=6, **Fig. 1B and 1C**). The printed cells started stretching along the longitudinal axis of the printed fiber structures at day 3 in culture, and the printed muscle structures induced the compaction phenomenon, keeping the fiber taut during cell differentiation. After 7 days of cell differentiation, a muscle-like structure with aligned myotube formation was observed. In *in vivo* study, implanted muscle constructs developed aligned myotubes or muscle fibers and maintained muscle characteristics (**Fig. 1D and 1E**), and blood vessels (**Fig. 1F**) and nerve ingrowth (**Fig. 1G**) into the implanted constructs were also determined.

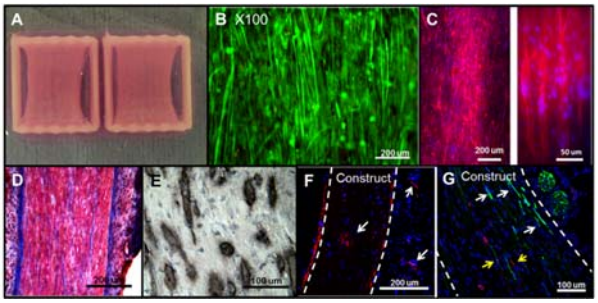

Figure 1. (A) Bioprinted skeletal muscle tissues fabricated by 3-D IOP system. (B) Live/dead assay and (C) MHC Immunostaining images of muscle constructs (red) at 7 days *in vitro* culture. Histological images of subcutaneously implanted muscle construct at 2 weeks; (D) Masson's trichrome, (E) MHC, (F) vWF (red), (G) NF (green)/AChR (red).

Conclusion

Our results demonstrate that creation of innervated volumetric engineered muscle tissues using the 3-D bioprinting system is feasible and that the functional muscle construct can contribute to restoration of muscle functions when applied to clinical translation.

References

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