Replacement Vascularized Skeletal Muscle for Craniomaxillofacial Reconstruction

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Introduction
In military and civilian settings, explosive devices cause severe injuries to craniofacial tissues. Small facial muscles, such as the orbicularis oculi (eyelid), experience extensive damage. Patients without functioning orbicularis oculi cannot close their eyelids and risk potential blindness. Allogeneic donor muscle transplantation represents the sole near-term therapy option, but requires life-long immunosuppression. Engineered autologous skeletal muscle will eliminate the risks associated with allogeneic sources but must be highly vascularised and innervated to be functional. We hypothesize that a vasculogenesis approach can be utilized to establish a prevascular network within engineered muscle tissue; this newly-created blood vessel network will readily anastomose with the host vasculature upon implantation.

Materials and Methods
3D muscles were engineered using a modified, previously reported methodology\(^1\). Primary myoblasts and fibroblasts were proliferated on fibrin gel anchored at two points. During differentiation, myotubes aligned between the anchor points. Fibroblasts degraded the fibrin and their contractile forces induced the co-culture to roll and self-assemble into 3D muscle tissue. The morphology and maturation of the engineered muscle was assessed using a range of assays. Microvascular networks were engineered within the muscle using a vasculogenesis approach\(^2\). Endothelial cells (human umbilical vein endothelial cells, HUVECs) and mesenchymal stem cells (MSCs) were cultured with myoblasts and fibroblasts, forming an endothelial-lined network within the muscle tissue. Vascularized engineered muscles were implanted into immunocompromised rodents. After 4 and 7 d, the muscle constructs were harvested and the vasculature was characterized using immunohistochemistry to identify endothelial markers.

Results
3D vascularized skeletal muscle tissue was successfully engineered. Engineered muscle has the characteristic interdigitated, multi-nucleated aligned architecture of native neonatal skeletal muscle, with sarcomere development clearly evident (Fig. 1). Prevascular networks were established within the muscle tissue by vasculogenesis. After 6 d of in vitro differentiation, a highly branched endothelial-lined vessel network formed, located on the muscle periphery (Fig. 2). Upon implantation, the engineered vessels anastomosed with the host vasculature and were perfused (Fig. 2C).

Discussion and Conclusions
Functional skeletal muscle must be vascularized and innervated. Neonatal skeletal muscle was engineered, which will mature to an adult phenotype upon innervation. Using vasculogenesis, an endothelial-lined network was established within the muscle in vitro which was perfused within 4d of implantation. Both are important milestones toward the development of replacement skeletal muscle for facial reconstruction.

References

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