

Skeletal Muscle Tissue Engineering In Patients With Facial Paralysis

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INTRODUCTION: Facial paralysis is a physically and socially disabling condition. Currently, conventional treatment strategies improve the quality of life of patients but remain suboptimal as these do not achieve regeneration. Innovative strategies based on regenerative medicine, in particular tissue engineering of skeletal muscle, are promising for treatment of patients with facial paralysis.

As a source for tissue engineering, myogenic satellite cells (SC) seem suitable. Satellite cells in skeletal muscles reside under hypoxic conditions. Moreover, development of skeletal muscle occurs under hypoxia. Therefore, hypoxia seems an important factor in tissue engineering of myogenic satellite cells. Our aims were to investigate whether hypoxic culture conditions would support tissue engineering of myogenic satellite cells derived from human muscle biopsies. And whether 3D organization of myotubes would be feasible for future *in vivo* experiments.

METHODS: Myogenic satellite cells are isolated from human muscle biopsies. They are cultured in normoxic (21% O₂) and hypoxic (2% O₂) conditions, and differentiation towards myoblasts and myotubes is initiated with low serum medium, ITS-A and dexamethason. Subsequently, the influence of hypoxic culture conditions on myogenic satellite cells is investigated through flow cytometry phenotyping, RT-PCR and immunofluorescent phenotyping. Furthermore, to generate 3D organized myotubes, culture regimens are optimized by culturing and differentiation of satellite cells in a fibrin gel.

RESULTS: In this study, CD56 and Pax7, markers for myogenic satellite cells, and other myogenic markers like MyoD1, Myf5, CKM, Myl1 and Myl3, indicative of myogenic differentiation, were expressed in both 21% and 2% O₂ cell cultures. Immunofluorescent microscopy revealed multinucleated myotube

formation and positive staining for myogenic markers like desmin (fig. 1), α -sarcomeric actin, and myosin heavy chain in both culture conditions. There was exponentially faster proliferation of myogenic cells under hypoxic culture conditions, important in reaching larger amounts of tissue for future clinical application. 3D organization of myotubes was reached in a fibrin gel (fig. 2).

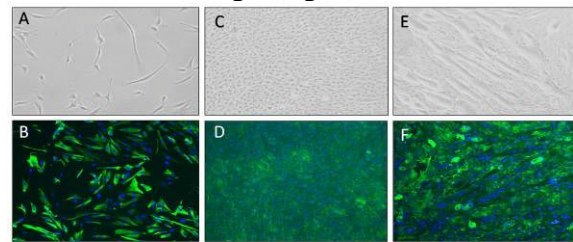


Fig. 1: Proliferating satellite cells (A) stained for desmin (B); Confluent culture of SC (C) stained for desmin (D); Differentiated SC form multinucleated myotubes (E) stained for desmin (F).

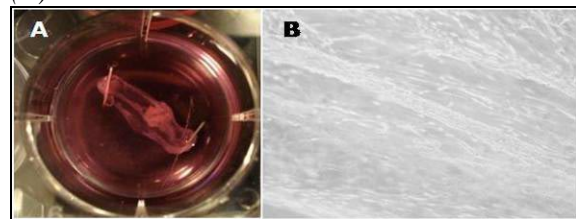


Fig. 2: SC in a fibrin gel. Macroscopic image (A); Microscopic image (B).

DISCUSSION & CONCLUSIONS: These results show a stimulating role of hypoxic culture conditions on proliferation of myogenic satellite cells. However, since more myogenic markers are expressed in cultures under 21% O₂, we state that for satellite cells to keep their myogenic properties ability to form myotubes, it is important to avoid oxidative stress.

3D organization of myotubes was reached in a fibrin gel, which makes further *in vivo* experiments feasible, and are the first steps towards tissue engineering of functional skeletal muscle from human myogenic satellite cells for treatment of patients with facial paralysis.