Muscle Regeneration and Function Restoration Following Transplantation of Chimeric Cells of Myoblast and Mesenchymal Stem Cell Origin

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BACKGROUND

Current clinical strategies to regenerate muscle tissue after massive tissue loss or muscular dystrophies have not shown significant efficacy. Allogeneic stem cell therapies and Vascularized Composite Allotransplants (VCA) aiming to restore affected muscles, are challenged by limited engraftment and rejection. Chimeric Cells (CCs), created via *ex vivo* fusion of donor and recipient cells, represent a novel and promising therapeutic option in the field of muscle regeneration and VCA, eliminating the need for life-long immunosuppression.
Myoblast (MB) and mesenchymal stem cells (MSC), with low immunogenic profile and regenerative characteristics, are optimal candidates for human Muscle Derived CC (MDCC) creation.

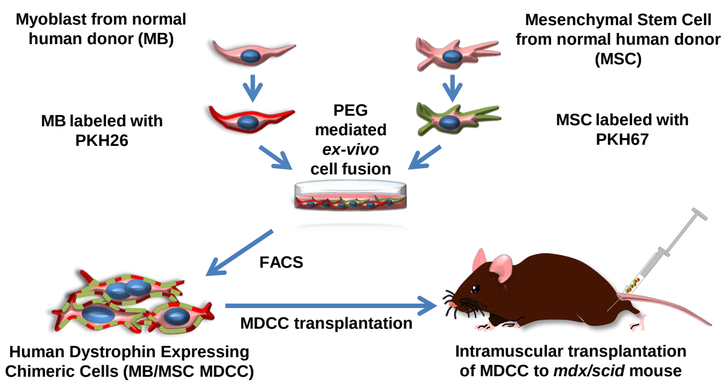
OBJECTIVES

- To characterize human MDCC *in vitro*
- To test the efficacy of MDCC engraftment *in vivo* as well as restoration of muscle function in the DMD *mdx/scid* mice model.

METHODS

Experimental Design

Schematic representation of fusion procedure



Sixteen *ex vivo* fusions of human MB-MSC were performed.

Experimental Groups

The engraftment of MDCC was tested in 27 mdx/scid mice, which received intramuscular injection of $0.5x10^6$ not fused MB and MSC or MB/MSC MDCC:

#	Group	Test article	N#	Route	Dose	Time point (days)
1	Control	Vehicle	3 and 6	IM.	0	7 and 90
2	Control	Not fused MB and MSC	3 and 6	IM.	$0.5x10^6$	7 and 90
3	Experimental	MB/MSC MDCC	3 and 6	IM.	$0.5x10^6$	7 and 90

Evaluation Methods

In vitro

I. Confirmation of fusion - confocal microscopy (CM) and flow cytometry (FC)
II. Dystrophin expression and myocyte differentiation– IF staining
III. Proliferation assay – FC
IV. Immunogenicity – Lymphocyte Proliferation Assay

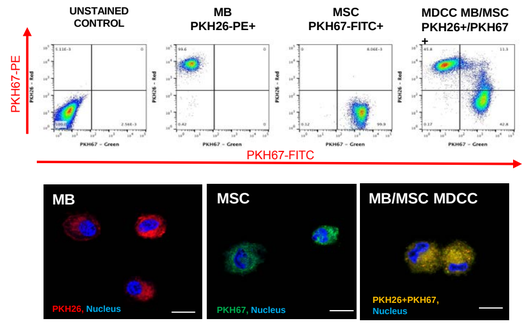
In vivo

V. Dystrophin expression– immunofluorescent staining
VI. Muscle function measurements:

•grip strength test,
•wire hanging test

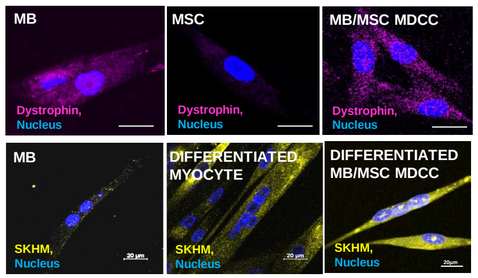
RESULTS

I. Confirmation of MDCC Creation by Flow Cytometry And Confocal Microscopy



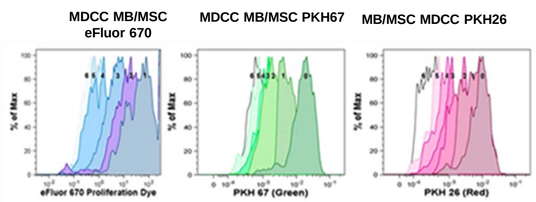
MB/MSC MDCC were FACSsorted based on their positivity for both PKH26 and PKH67. Fusion was confirmed by fluorescence analysis with confocal microscopy. MDCC were observed as orange cells (overlapping PKH26 and PKH67, magnification 400, scale bar 20µm).

II. Expression of Dystrophin and Skeletal Myosin Heavy Chain (Differentiation Marker) by MDCC



MB/MSC MDCC express dystrophin after fusion procedure.
Following a 7-day culture in specific myogenic differentiation media, MB/MSC MDCC expressed mature myocyte marker (skeletal heavy chain myosin-SKHM) and underwent spontaneous myotube formation (magnification 400, scale bar 20µm).

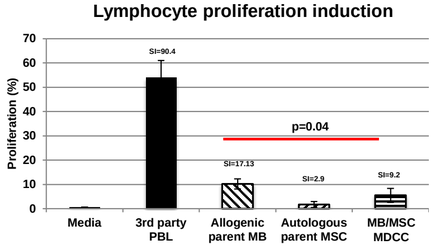
III. MDCC Proliferation Assay



Proliferation of MDCC was confirmed up to 21 days after fusion by proliferation dye (blue-eFluor 670). eFluor 670 proliferation results correlated with proliferation detected with PKH67 and PKH26 membrane dyes.

IV. MDCC Induced Lymphocyte Reactivity

Lymphocyte proliferation induction

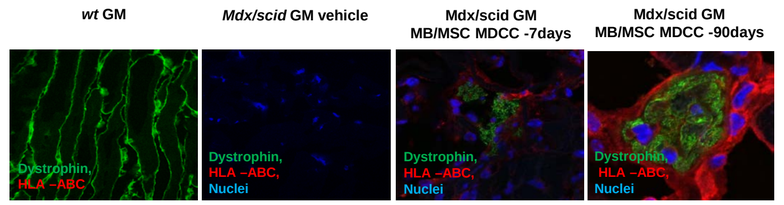


Flow cytometry proliferation assay (eFluor 670) tested reactivity and proliferation of MSC (parent cell) donor derived lymphocyte after a four-day co-culture with media (negative control), 3rd party lymphocytes (PBL, positive control), allogeneic parent MB, autologous parent MSC and MB/MSC MDCC .

Proliferation is expressed in percentage and by stimulation index (SI, proliferation count normalized with negative control values).
MB/MSC MDCC reduced lymphocytes proliferation in respect to allogeneic MB parent cell confirming low-immunogenicity of MDCC.

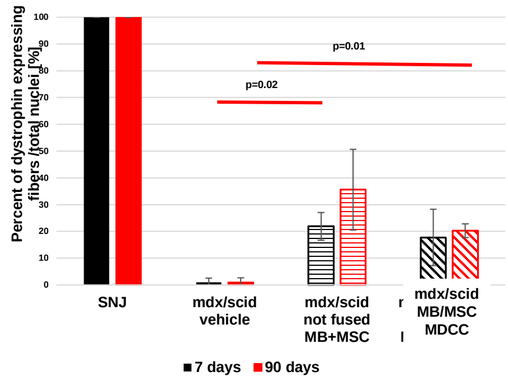
RESULTS

V. Dystrophin Expression in the Treated Gastrocnemius Muscle After Local MB/MSC MDCC Injections



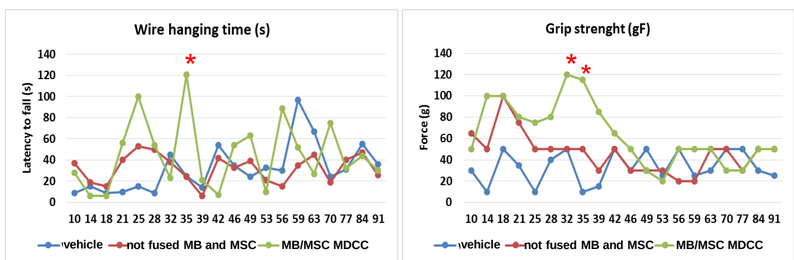
MB/MSC MDCC engraftment were observed at 7 and 90 days after MDCC delivery. Human origin of dystrophin-positive fibers were confirmed by co-localization of HLA-ABC staining, which also excluded the involvement of revertant fibers.

Quantification of dystrophin expressing fibers



At day 7, 17.7 % increase in dystrophin expressing fibers was observed in MB/MSC MDCC treated animals compared to vehicle control. At day 90, 20.26% increase of dystrophin expressing fibers was preserved in MB/MSC MDCC treated groups.

VI. Muscle function evaluation *in vivo*



CONCLUSIONS

- This study confirmed feasibility of myoblast and MSC fusion and the created MB/MSC MDCC showed successful engraftment and restoration of dystrophin in affected muscles.
- Muscle function improvement were shown in muscle strength tests.
- MDCC therapy represents a novel, universal approach for restoration of muscle function in muscular dystrophy, traumatic muscle tissue loss and regeneration of muscle components of the VCA.