Expanded Muscle Derived Stem Cells-Chitosan Sheet Trasplantation for the Treatment of Muscular Dystrophy
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Introduction
Loss of functional skeletal muscle caused by traumatic injury, congenital defects or functional damage due to a variety of myopathies produces a physiological deficit for which there is still no effective clinical treatment. Transplantation of exogenous myogenic cells (satellite cells and myoblasts) has been proposed to increase the regenerative capacity of skeletal muscle. However, the clinical outcomes from intramuscular injection of allogeneic myoblasts is hampered by numerous limitations, including poor cell retention and survival, as well as immunorejection.

Current interest in regenerative medicine have prompted the development of living cells and polymeric materials (scaffold), which are crucial for the successful regeneration of tissues and organs.

We investigated an alternative method for transplanting expanded muscle-derived stem cells by using a natural polymeric membrane. We test this approach using muscle-derived CD133+ stem cells in a murine model of Duchenne muscular dystrophy, the scid/mdx. In particular we verified whether the in vivo myogenic regenerative potential of these stem cells should be improved using a chitosan scaffold as support for tissue transplant.

Materials and Methods
Chitosan membrane (2% w/v) were obtained by using solvent casting technique under laminar flow. Film surfaces were observed under Scanning electron microscopy (SEM) and profilometer. Muscle derived stem cells were seeded onto the scaffolds, pre-soaked overnight in culture medium. Xenogenic transplantation studies were performed using scid/mdx mouse model injected with the cell-material substrate and control cells. We performed in vitro and in vivo immunofluorescence staining in order to analyse in vitro and in vivo stem cell survival and differentiation.

Results
SEM and profilometer analysis showed a very smooth surface of range roughness 5nm and 100 μm of thickness. The CD133+ stem cells cultured on chitosan scaffold well maintain their capacity to differentiate into myogenic cells expressing human desmin. No differences in term of myogenesis was observed comparing CD133+ stem cells cultured on dish and CD133+ stem cells cultured on chitosan scaffold. By this way we tested the transplantation of muscle-derived CD133+ stem cells seeded on the chitosan scaffold in vivo. The results of these experiments demonstrated the biocompatibility of chitosan scaffold/CD133+ expanded cells in scid/mdx mice. In fact 30 days after the implant of the chitosan/CD133+ stem cells we didn’t observe any immune-reaction in host muscle tissue (not presence of CD4 or CD8 positive cells or inflammatory cells showing the macrophages markers) and the injected cells were proliferating and vital, without apoptotic evidences (data not shown). Muscle-derived CD133+ cells well migrate from the chitosan film (Fig 2a) and fuse with the host myofibers expressing human myosin developmental (Fig 2b). Moreover the transplanted cells were also able to differentiate in cells expressing CD31 and von Willebrand Factor (vWF) human endothelial markers. By these data we appreciate a better migration and differentiation capacity of the muscle derived CD133+ stem cells by using a chitosan film for their transplantation.

Fig.1 SEM image of the membrane surface
Fig.2 In vivo desmin (fig2.a) and human myosin developmental (fig2.b) immunostaining on muscle-derived stem cells-chitosan substrate.

Discussion and Conclusions
We showed that tissue engineering approach with CD133+ cells on chitosan film is a promising tool for a future application in regenerative medicine to treat muscular diseases. Further studies are needed to improve the efficiency of endothelial/myogenic differentiation capacity of transplanted muscle-derived CD133+ stem by the use of different types of scaffolds as support for the cell transplantation.

References

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