AMNIOTIC STEM CELLS SHARE CLUSTERS OF DIFFERENTIATION WITH FIBROBLASTS AND KERATINOCYTES AND INFLUENCE THEIR ABILITY TO PROLIFERATE AND WOUND HEALING (120)

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Introduction: Commencing fibroblasts and keratinocytes cell cultures for severely burned patients is very problematic due to low viability and number of cells isolated from donor skin. These cultures last long due to the high doubling time. Number of obtained cells is often insufficient to cover the extensive burn wounds, and the waiting time for culturing slows down the healing process. The solution to this problem is permanently available source of allogeneic cells, which are easy to isolate and culture, with a high number of population doubling, showing no senescence in spite of repeated passages and able to differentiate into keratinocytes and fibroblasts. Considerable source for these purposes should be human amnion. Transplants of these cells should be performed as soon as possible after defining the depth of burns in order to stimulate the healing process while waiting for end of culture of autologous fibroblasts and keratinocytes in order to improve the quality of wound healing. Moreover, due to the immunosuppressive properties amniotic cells can be used with allogeneic transplants fibroblasts and keratinocytes. The aim of this study is to verify ability of human amniotic mesenchymal stem cells (hAMMSCs) to share cluster of differentiation with fibroblast and keratinocytes in order to support autologous or allogeneic cells transplant and to verify impact of hAMMSCs on keratinocytes and fibroblasts ability to proliferation and wound healing.

Methods: Amnions were derived from 3 donors who provided written consent. Fibrolbasts and keratinocytes came from the Tissue Bank. To study the similarity of cluster of differentiations (CD) markers such as CD26, CD13, CD10, Integrin alpha-1, CD39, FSP, bFGF, Vimentin, CD98, Keratinocyte Transglutaminase, KGF, Thrombomodulin, Pormin, TNFRSF5. In order to differentiate the cells were used: cell culture medium for differentiated cells, Boyden dishes and co-culture. Analysis of CD were performed at 4, 8 and 12 day of culture. The controls consisted of cultured amniotic cells using a standard medium (AmnioPan), and fibroblasts (DMEM with FBS) and keratinocytes (KGM-CD). We also examined the impact of amniotic stem cells on fibroblasts and keratinocytes ability to close wounds in vitro (Wound Healing Assay) and proliferation rate (Click-iT® EdU Alexa Fluor® 488 Imaging Kit).

Reluts: Statistical analysis showed that there is no significant differences between the levels of surface markers depending on the method of differentiation compared to fibroblasts and control ($p \geq 0.05$). Presence of all the surface markers of keratinocytes was also detected. Moreover it was found that there is a difference between the proliferation rate of fibroblasts and co-cultures of fibroblasts and amniotic stem cells (p

Conclusions:
1. hAMMSCs stimulate fibroblast proliferation rate and the ability of fibroblasts and keratinocytes wound closure.
2. hAMMSCs have the full panel of fibroblasts and keratinocytes tested cell markers.
3. hAMMSCs can be used as first-line transplantation or assist both allogeneic and autologous transplantations.