ADULT MESENCHYMAL STEM CELLS ROLE IN HEALING PROCESSES

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MATERIAL AND METHODS

Adult mesenchymal stem cells (MSCs) could be the election choice for regenerative medicine due to their ability to differentiate towards various cellular lineages.

We performed a comparative study (bone-marrow MSCs and dental pulp MSCs) aiming to demonstrate the cells capacity to stimulate de wound healing processes.

Mesenchymal stem cells isolation

• Bone-marrow derived MSCs were harvested by sternal punction from 4 patients suffering severe anaemia

• Dental pulp derived MSCs were isolated from 3 patients undrgoing tooth extraction

•All human samples were obtained after signing the informed consent elaborated under an approved protocol, according to the World Medical Association Declaration of Helsinki

•MSCs expanded to passages 2-5 were used in the subsequent experiments

Epithelial differentiation of MSCs

• DMEM low glucose (1 g/l) +
• 10% FCS +
• 10 ng/ml KGF +
• 20 ng/ml EGF +
• 10 ng/ml HGF +
• 60 ng/ml IGF-2

Evaluation of the differentiation process after 21 days by citokeratin, E-cadherin and EpCAM expression

In vitro functional studies

1. In vitro secretory profile was determined using ELISA test

2. Flow chamber adhesion assay:

• fibronectin, laminin and collagen type I were used as coating in 6-channels µ-Slide VI ibiTreat flowchamber

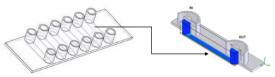
• 100 μl of 100,000 MSCs were left to interact with the substrate for 3 minutes

• increasing shear stress of 0.35, 2, 5, 8 and 15 dynes/cm^2 was generate for 3 minutes each

• pictures of the centered microscopic field were taken every 30 seconds, for every value of shear stress

• total cell count was compared with the control

• variations of at least 15% in cell count were considered signific



3. In vitro scratch-assay

• the cells were cultured on different types of coating: laminine, fibronectine and matrigel membrane matrix

• a wound was induced in a cell monolayer (15 mm wide scratch)

• the cells were treated with 1ng TNFα /FGF - 24 hours

• the cells capacity to repair the wound was assessed at 8h, 24h, 48h and 72h

Animal experiments

12 B6D2F1 female mice, 75 ± 5 g weight, skin lesions (0.5 cm^2)

• control group (n=4) - healing process per se
• 2 experimental groups
  - supplementary immunosuppressed 3 weeks before (i.p. 100 µl Dexamethasone, 4 mg/ml)
  - 10^5 cells/100 µl injected at the injury site
    - undifferentiated MSCs (right)
    - MSCs induced into epithelial-like cells (left)
  - group I (n=3) treated with bone marrow derived MSCs
  - group I (n=3) treated with bone marrow derived MSCs

• when the healing process finished (formation of uniform cornous layer), the animals were sacrificed and the newly formed epidermal layer was harvested

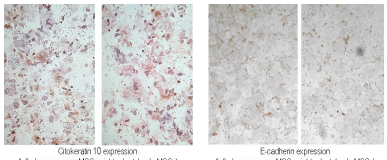
• comparative evaluation of the healing process: hematoxylin-eosin staining of 3 µm thick sections

•investigations for expression of the human proteins of interest: Vimentin, E-cadherin, Integrin beta 1

All animal experiments comply with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Directive 86/609, Strasbourg, 1986) and the experimental protocol was reviewed and approved by the University of Medicine and Pharmacy Timisoara Board for Animal Experiments

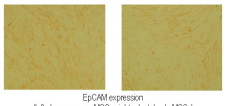
RESULTS AND DISCUSSIONS

MSCs induction in epithelial like cells



Citokeratin 10 expression
(left - bone marrow MSCs, right - dental pulp MSCs)

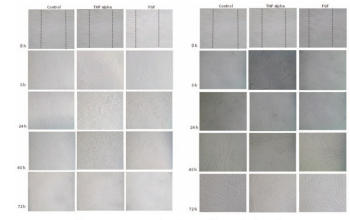
E-cadherin expression
(left - bone marrow MSCs, right - dental pulp MSCs)



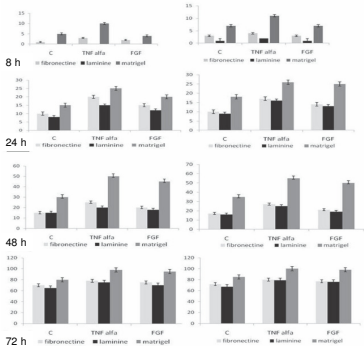
EpCAM expression
(left - bone marrow MSCs, right - dental pulp MSCs)

In vitro scratch assay

High closing wound percentage was obtained for cells cultivated on matrigel and TNFa prestimulated, without significant differences regarding the MSCs source.



The most representative images for matrigel coating induced wound scratch (right - bone marrow MSCs, left - dental pulp MSCs)



Wound closure percentage of TNF-alpha and FGF treated dental pulp MSCs (left) and bone-marrow derived MSCs (right) after performing the wound scratch

Secretory profile of MSCs

• Human MSCs release in culture media high concentrations of VEGF, TGFbeta and TNFalfa, demonstrating that this cells have immunomodulatory properties.

• Epithelial induction do not determine significant changes of secreted cytokines

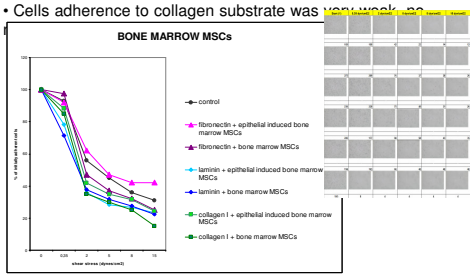
• There are no differences between bone marrow derived a

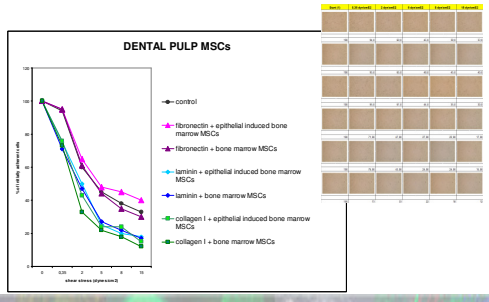
	IL-13 (pg/ml)	IL-4 (pg/ml)	TGF β (pg/ml)	VEGF (pg/ml)	IL-2 (pg/ml)	IL-10 (pg/ml)	IL-12 (pg/ml)	TNF α (pg/ml)	INF γ (pg/ml)
Bone marrow MSCs	0,245	0,211	2823,35	145,56	1,657	1,25	0,285	7,546	5,127
Dental pulp MSCs	0,194	0,125	2737,451	111,23	1,954	0,854	0,129	8,652	3,965
Epithelial induced bone marrow MSCs	0,231	0,102	2603,215	134,59	2,25	0,742	0,387	7,253	5,236
Epithelial induced dental pulp MSCs	0,246	0,037	2513,547	168,57	1,981	0,657	0,258	7,489	4,872

Flow chamber adhesion assay

• An increased adherence of epithelial-induced cells to fibronectin was noticed without differencies regarding the MSCs source.

• Cells adherence to collagen substrate was very weak, no

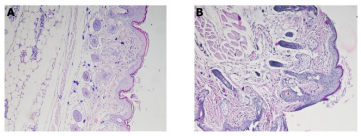




Murine experimental model

• Injection of epithelial-like differentiated MSCs in both experimental groups induced a faster healing of the injury in 10 days compared with the control group in which the epidermal injury healed in 15 days *per se*

•Injection of undifferentiated hMSCs in both experimental gr



A. Treatment with epithelial induced MSCs: the epidermal layer is continuous with normal aspect

B. Treatment with undifferentiated MSCs: the re-epithelization is disturbing the normal structure of epidermal components

A.Intense membrane staining for E-cadherin is shown after treatment with epithelial-induced MSCs. **B.**Human Vimentin stained positive (brown) within the cytoplasm of undifferentiated hMSCs injected into the group II. Cells were located at the level of murine pilous bulbs, demonstrating migration of stem cells towards their own niche. **C.**Mesenchymal derived human cells expressing beta1-integrin (red) likewise localised near the pilous bu
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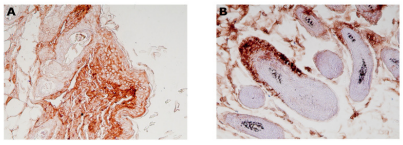

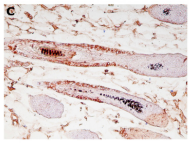

Immunohistological analysis of engrafted human MSCs (magnification 200x).

Presence of human structural protein was revealed by staining with human specific primary antibody, which showed no cross-reactivity to mouse proteins.

CONCLUSIONS

The changes in migration and adhesive properties suggest that MSCs from both sources are activated in response to wounding.

Induction of MSCs into epithelial lineage seems to increase the cells efficacy in the in vivo tissue-restoration procedures.

Present study provides strong evidence that epithelial-differentiated MSCs induce a faster healing of the epidermal lesions, engrafting at the level of differentiated cells layer in immunosuppressed murine models.

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