THE MESENCHYMAL STEM CELLS BIOENGINEERED TISSUE USING COBALT-60 RADIATION.

MOSCA, RC., ISAAC, C., MATHOR, MB.

Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP),
Av. Prof. Lineu Prestes 2242
CEP 05508-000 São Paulo, S.P.
rcmosca@usp.br

ABSTRACT

The biological effects of gamma radiation by cobalt-60 sources on epidermal-dermal matrix have not been fully elucidated when mesenchymal stem cells cultured add in. We propose an experimental model where the replenishment of the depleted stem cell compartment of human skin may be explored using a dermal epidermal equivalent system, composed by 50 Gy irradiated extracellular collagen matrix, adipocytes derived mesenchymal stem cells (ADSCs) and normal keratinocytes. The histological Hematoxylin-eosin stained samples showed keratinocytes stratified over one of the matrix face while ADSCs invaded the collagen network, the removal of all basal membrane components during preparation of the dermal support also provided the reorganization of the dermal-epidermal junction. Thus, suggesting the possible use of this engineered compound as a permanent skin substitute.

MATERIAL AND METHODS

- **Standardization of keratinocytes and adipose derived stem cell (ADSC) culture procedures**

Biological tissues were obtained from skin grafts (keratinocytes) and fat (ADSC) from the Hospital das Clínicas Tissue Bank of São Paulo University.
Devoid of subcutaneous tissue by serial enzymatic cell separation using an 0.05% trypsin/0.02% EDTA solution. Cell cultures were fed initially with a mixture of 60% Dulbecco's Modified Eagle's Medium (DMEM), 30% Ham F12, and 10% fetal bovine serum (FBS), supplemented with 4 mM Lglutamine, 0.18 mM adenine, 5 g/ml insulin, 0.4 g/ml hydrocortisone, 0.1 nM cholera toxin, 2 nM tri-iodothyronin and 100 IU/ml penicillin/100 g/ml streptomycin antibiotic solution. Keratinocytes were maintained at 37° C and 5% CO_2 atmosphere and media were changed each 48 hours and cell proliferation was observed in optical microscope. Keratinocytes were seeded over 3T3 feeder layer.
For ADSC differentiation in adipocyte, these cells were cultured inside culture flasks of 25 cm^2 surface area in $DMEM_{10}$ added with indomethacin, theophylline, insulin and dexamethasone. ADSC cultures began to show cell differentiation after two day of special medium presence. ADSC were maintained at 37° C and 5% CO_2 atmosphere and media were changed on 48 hours and 168 hours and cell differentials was observed in optical microscope.

- **Construction of a bioengineered dermal-epidermal equivalent populated by keratinocytes and adipose derived stem cell (ADSC) cultured cells**

Fresh split thickness skin grafts obtained from the Hospital das Clínicas Tissue Bank (minimum of 85% glycerol solution). Epidermal cells were removed by placing precut 2 cm × 2 cm fragments in a thermically sealed polyethylene packaging and underwent 50 Gy radiation dose. For rehydration of the sample, the glycerol is removed from the tissue by 3 baths in sterile saline 0.9% for 20 minutes. Epidermal cells were removed placing 2.5 mg/ml dispase solution for 90 minutes at 37° C, followed by a second 15 min bath in an 0.05% trypsin/0.02% EDTA solution, also at 37° C.
An immerse and air-liquid interface culture systems was tested. This cultures system were started by plating approximately 60,000 cells/cm^2 of each cell type on dermis in culture, immersing completely the dermis. The first medium change was performed after 24 h and then every second day for the next 2 weeks. From the fourteenth to the twenty-first day of the experiments, culture medium was changed daily. When an air-liquid interface situation was required for sample containing keratinocytes and ADSC, after culturing in the immerse situation for 7 days, the dermal fragments with proliferating cells were elevated onto metal grids, and enough culture medium was added to the well to keep the dermis in contact with the medium and the cells exposed to air.

RESULTS

- **ADSC differentiation in adipocyte**

ADSC cultures showing cell differentiation after seven days of special medium presence (Figure 1).

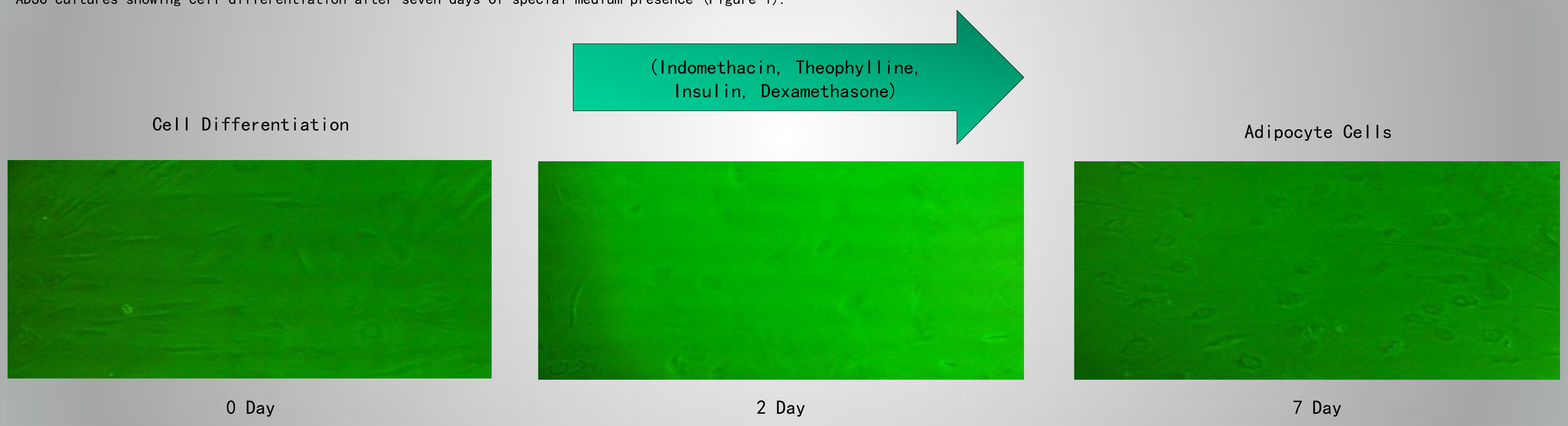

Figure 1 - ADSC differentiation into adipocytes after seven days (M.O. with yellow filter - 400x)

- **Bioengineered dermal-epidermal equivalent populated by keratinocytes and adipose derived stem cell (ADSC)**

Immerse and Air-Exposed culture systems of dermal-epidermal composites matrix repopulation with keratinocytes (Figure 2) and ADSC with keratinocytes (Figure 3 and 4).

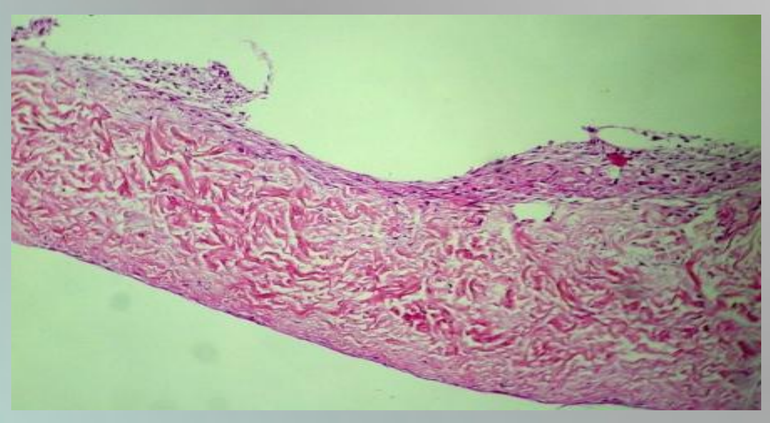

Figure 2 - Keratinocytes seeded over a 50 Gy irradiated dermal matrix an immerse systems after 21 days (M.O.- 100x - H.E)

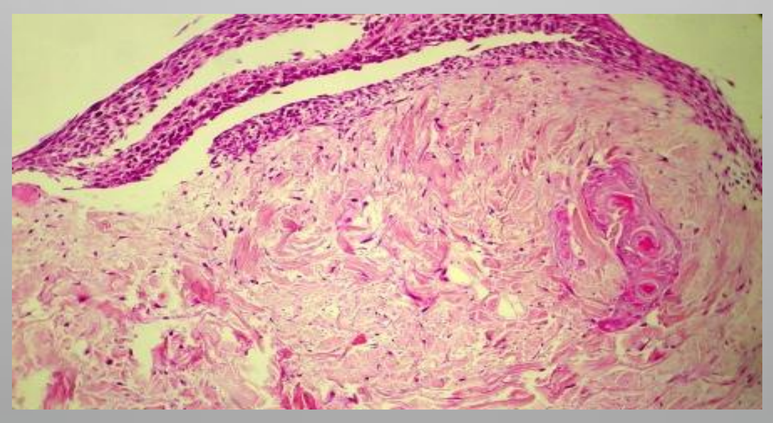

Figure 3 - Keratinocytes and ADSC seeded over a 50 Gy irradiated dermal matrix an air-liquid culture systems after 21 days (M.O.- 100x - H.E)

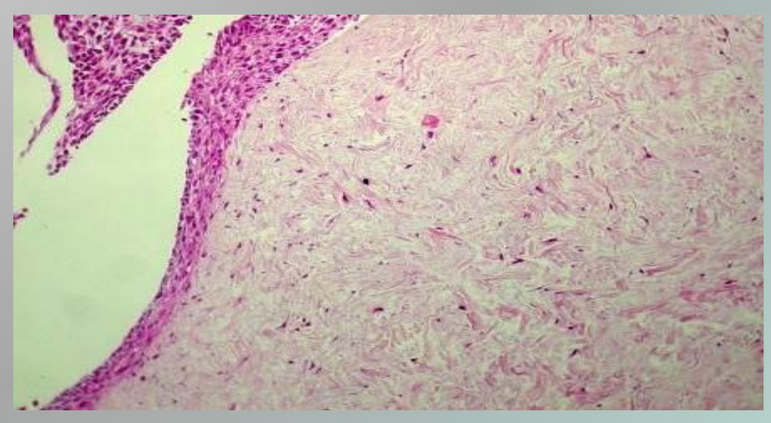

Figure 4 - Keratinocytes and ADSC seeded over a 50 Gy irradiated dermal matrix an air-liquid interface culture systems after 21 days (M.O.- 400x - H.E)

CONCLUSIONS

Nowadays the use of skin equivalents as a graft is a reality. The new challenge is how to improve those skin equivalents performances by adding stem cells. In our partial results we have demonstrated keratinocytes and ADSC capacity of repopulate an acellular human dermal matrix.

ACKNOWLEDGMENTS

This work was prepared with financial support from the IAEA through the RC project (Research Contract No. 15333), with the support of CNPq Universal Project - 480390/2010-0 and CNEN to grant the doctoral scholarship for the student Rodrigo Crespo Mosca.