# New Defined Cell Culture Conditions in Combination with a 3D-Scaffold for MSCs Bone Tissue Engineering

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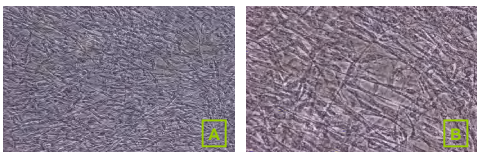
## Introduction:

Skeletal tissue loss due to congenital defects, disease, and injury is normally treated by autologous tissue grafting, a method limited by the availability of the host tissue, harvesting difficulties, donor site morbidity, and clinician's ability to manipulate delicate 3D shapes.
The generation of autologous bone grafts *in vitro* avoiding the harvesting of autologous tissue at a second anatomic location is the ultimate goal in bone tissue engineering. We have developed a protocol for the isolation and culture of human adipose tissue-derived mesenchymal stem cells (AT-MSCs) which fulfils the strict European regulations concerning the Advanced Therapy Medicinal Products. AT-MSCs were grown inside a new 3D-scaffold based on equine bone grafts. This scaffold allows to maintain the adequate 3D-structure, promote cell adhesion and proliferation. The osteogenic differentiation of human AT-MSCs was obtained by an animal-free induction medium without the use of growth factors.

## Methods:

Human AT-MSCs were obtained from liposuction aspirates, cultured and expanded using protocols developed in our laboratory. After seeding the AT-MSCs into the scaffold (provided by Osteoxenon, Italy), the cells were cultured for three weeks in presence of a defined osteogenic induction medium optimized for clinical use. The bone chips were then fixed and analyzed by histochemical methods.




CONTROL – NOT INDUCED: A. 10X magnification; B. 20X magnification.

***Fig.2. Control and osteogenic induced MSCs.*** To determine whether our osteogenic induction medium had any effect on cell morphology or proliferation, control and induced Petri dishes at the same cell concentration of the bone scaffold experiment were cultured in parallel. Cell morphology changed deeply already at day 5 after osteogenic induction (20X magnification).

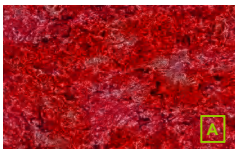
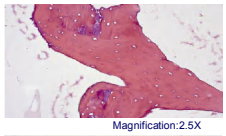



***Fig.3. Cells grew in our new defined cell culture medium show great osteogenic and adipogenic differentiation potential.*** A & B. Osteogenic induction after 21 days shows extensive calcium deposition as demonstrated by positive Alizarin Red S staining. C. Adipogenic induction as demonstrated by marked lipid droplets deposition stained with Oil Red O. Defined ("animal-free") cell culture media were used for both in vitro induction assays.

A: Osteo-induction, 10X magnification. B: Osteo-induction, 20X magnification. C: Adipo-induction, 10X magnification.

***Fig.4. Ematossilin/Eosin staining on osteo-induced bone chips.*** Bone chips with osteo-induced adherent MSCs were decalcified, paraffin-embedded, cut into slices and stained for HE. Cell nuclei are apparent on the bone chips.



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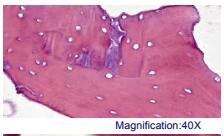
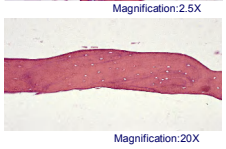
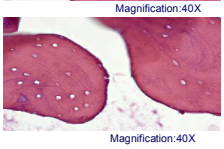


Magnification:40X



Magnification:20X



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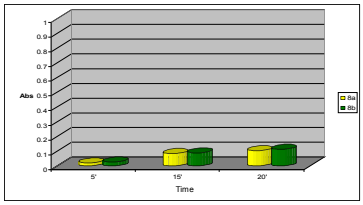


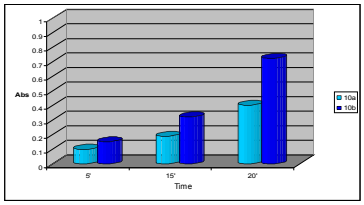
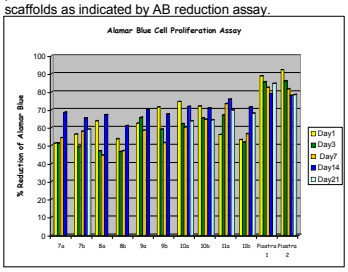




***Fig.5. Alkaline Phosphatase (ALP) assay.*** ALP activity increases with time in induced cellularized bone scaffolds. Elevated ALP activity indicates that there is active bone deposition occurring as ALP is a byproduct of osteoblast activity.

***Fig.6. Alamar Blue Cell Proliferation assay.*** Cell proliferation increases with time on cellularized bone scaffolds as indicated by AB reduction assay.




## Results:

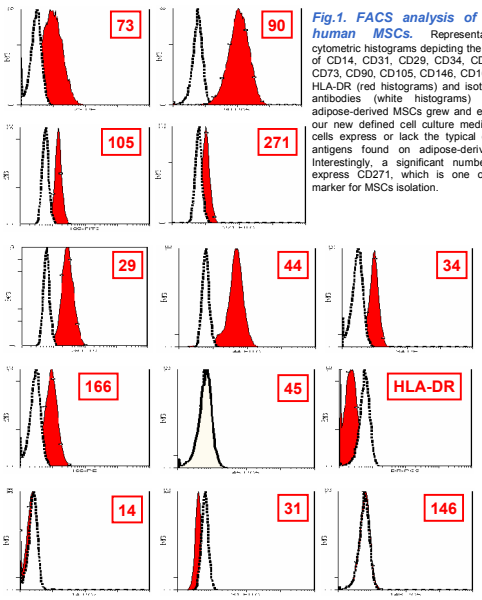



***Fig.1. FACS analysis of cultured human MSCs.*** Representative flow cytometric histograms depicting the expression of CD14, CD31, CD29, CD34, CD44, CD45, CD73, CD90, CD105, CD146, CD166, CD271, HLA-DR (red histograms) and isotype control antibodies (white histograms) in human adipose-derived MSCs grew and expanded in our new defined cell culture medium. These cells express or lack the typical cell-surface antigens found on adipose-derived MSCs. Interestingly, a significant number of cells express CD271, which is one of the best marker for MSCs isolation.

## Conclusions:

A biocompatible 3D-scaffold, in combination with our clinically optimized culture conditions, appears to be very suitable for the growth and differentiation of human adipose-derived MSCs into osteogenic cells. Hystochemical and Real-Time PCR analysis is ongoing to refine our results.
This strategy involving an *in vitro* generated bone graft seems very promising for the clinical treatment of bone loss or injury.