

## Engraftment of human amniotic fluid stem cells (AFSCs) in calvarial bone of immunodeficient mice

Francesco Marchegiani<sup>1</sup>, Lucia Centurione<sup>1</sup>, Maria Antonietta Centurione<sup>2</sup>, Alexander Lichtler<sup>3</sup>, Ivo Kalajzic<sup>3</sup>, Roberta Di Pietro<sup>1</sup>

<sup>1</sup> Department of Medicine and Ageing Sciences, G. d'Annunzio University, 66100 Chieti, Italy

<sup>2</sup> Institute of Molecular Genetics, National Research Council, 27100 Pavia (Section of Chieti), Italy

<sup>3</sup> Department of Reconstructive Sciences, University of Connecticut Health Center, 06030 Connecticut, United States

AFSCs represent an attractive cell model for transplantation therapy due to the lack of significant immunogenicity, tumorigenicity and ethical issues (De Coppi et al., 2007). Although AFSCs have been investigated for bone repair, the cellular distribution and post-implantation viability remain key issues (Dupont et al., 2010). The present study was aimed at investigating whether AFSCs could improve bone healing in a calvarial defect model using immunodeficient mice. For this purpose AFSCs were transfected with a lentiviral vector expressing a ubiquitously directed red fluorescent protein-cherry. For *in vivo* experiments a critical size (3.5 mm) calvarial defect was developed in NOD scid gamma (NSG) immunodeficient mice. Human AFSCs were expanded *in vitro* and transfected at the 1st passage, then transplanted *in vivo* at the lesion sites after being loaded on HEALOS® scaffold (cross-linked collagen fibers fully coated with hydroxyapatite) appropriately shaped to cover the bone lesion. The calvarial defect was filled with the scaffold alone in control mice. Six weeks after implantation all animals were subjected to a skull X-ray before being sacrificed. Calvarial bone specimens were fixed in paraphormaldehyde, cryopreserved with sucrose and embedded in Cryomatrix TM resin. Sections were observed under fluorescence microscopy to detect the cherry-red signal, and then stained with haematoxylin-eosin solution to better analyze histological structures. Radiography scans of *ex vivo* bone explants demonstrated the presence of qualitatively and quantitatively mineralized tissue levels in the defect. Light microscopy observations revealed a major fibrous reaction in mice specimens treated with the scaffold supplemented with AFSCs compared with mice treated with the cell-free scaffold. The presence of cherry-positive AFSCs was recognized in the newly formed fibrous bone often around the scaffold and close to newly formed vessels. Our findings indicate that undifferentiated AFSCs seeded on a collagen scaffold can engraft in a host bone contributing to new bone and vessel formation. These preliminary observations pave the way to the use of new bioengineered constructs of stem cell-collagen scaffold for correcting large cranial defects in animal models and human subjects.

### References

- [1] De Coppi P et al. (2007) Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 25(1): 100-6.
- [2] Dupont KN et al. (2010) Human stem cell delivery for treatment of large segmental bone defects. *PNAS* 107(8): 3305-10.

### Keywords

AFSCs, transfection, Cherry red-fluorescent protein, bone defect.