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

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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

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