

Regenerative properties of human embryonic stem cell-derived chondrogenic cells in an articular defect.

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INTRODUCTION: Cartilage possesses limited self-renewal potential, therefore any damage to this tissue, chronic or acute, such as those arising from sports injuries, osteoarthritis and rheumatoid arthritis will eventually result in loss of cartilage with possible exposure of the subchondral bone and resultant pain and immobility. Strategies for the assisted repair of skeletal tissues are urgently required to alleviate the high morbidity associated with joint disorders. The World Health Organisation (WHO) has shown that the burden to society of these disorders is not only significant in terms of absolute disability-adjusted-life years (DALYs) but that it is seen (and is growing) in both the developed and developing world.

Current therapies are restricted by shortage of grafting material, insufficient biocompatibility of the implanted material and absence of cells with reparative potential. The unique biological properties of human embryonic stem cells (hESCs) (Thomson *et al.* 1998) highlight them as ideal candidate cell sources for such therapies by virtue of their directed differentiation potential and their ability to undertake large scale proliferation for bulking up prior to use.

The aim of this study was to produce hESC-derived chondroprogenitor cells and to assess their regenerative properties in an articular cartilage defect in the rat knee.

METHODS: Cells from a registered hESC line were expanded in feeder free conditions using established techniques. Embryoid body-derived cells were cultured using an in-house defined media system capable of inducing chondrogenic differentiation to form 3D cell constructs. Markers of chondrogenic differentiation were monitored in constructs prior to use experimentally. Pre-labeling with the fluorescent cellular probe CM-Dil (20 μ M; Molecular ProbesTM) allowed tracking of implanted human cells in the rat defect. Defects were created surgically on the femoropatellar groove of the knee joint of rats and the constructs were implanted. Empty defects served as controls.

Samples were collected 21 days post-implantation and snap frozen. Fixed cryosections were assessed both morphologically and histologically for repair and to track the persistence of implanted human cells. A subset of constructs were collected at the time of implantation to enable characterisation of implanted material.

RESULTS: HESC-derived constructs exhibited a chondrogenic phenotype at the time of implantation as shown by IHC and gene expression of articular cartilage specific Collagen II. Following 21 days implantation, the regenerate tissue not only showed good integration with the host tissue but also a hyaline cartilage-like morphology and excellent values when scored using a modified system based on Wakitani *et al.* (1994) and Horaf (2003) for markers of healthy cartilage (Fig 1). In comparison, empty defects showed poor repair with a fibrocartilaginous tissue filling the defect area. The presence of CM-Dil labeled cells within the defect area demonstrated the persistence of human donor cells (Fig 2).

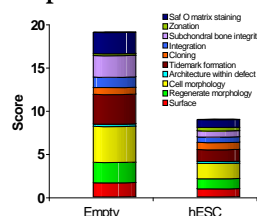


Fig 1: Histological grading of regenerate tissue within defect

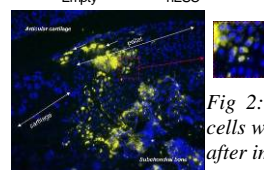


Fig 2: Detection of CM-Dil labeled human cells within repair tissue in the defect 21 days after implantation

DISCUSSION & CONCLUSIONS: This study points to the future use of hESC-derived chondrogenic cells in the treatment of cartilage defects in man. The ultimate aim of our work is to address the huge disease burden associated with osteoarthritis and future studies will focus on ways to support the chondrogenic cells in the harsh OA environment.

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