Introduction

Cell-based tissue engineering offers a promising means of replacing or repairing lost or damaged tissues. Osteoarthritis (OA) is the most prevalent of all musculoskeletal diseases, characterised by the progressive destruction of articular cartilage. As mature articular cartilage has a very limited regenerative potential, treatment options for OA are limited and ultimately the disease leads to joint arthroplasty. Adult mesenchymal stem or stromal cells (MSCs) contribute to the maintenance of connective tissues and local delivery of MSCs to the injured joint in a goat model of OA retarded the progressive destruction of cartilage (1). Transplantation protocols for delivery of human MSCs (hMSCs) have, in general, resulted in poor engraftment which may be associated with apoptosis. Thus, the introduction of an apoptosis inhibitor such as Bcl-xl may hold therapeutic promise as a means of expanding the lifespan of transplanted hMSCs. The objective of this study was to evaluate the effect of Bcl-xl overexpression on hMSCs and to examine the survival and chondrogenic potential of Bcl-xl hMSCs to promote tissue repair in a human OA cartilage explant model.

Materials and Methods

Isolation and Expansion of hMSCs and Human Cartilage Explants:

Bone marrow-derived hMSCs were obtained from the iliac crest of normal donors and cartilage explants were obtained from OA patients undergoing total knee arthroplasty. All procedures were performed in accordance with informed consent and approved by the Clinical Research Ethical Committee at University College Hospital, Galway. hMSCs were isolated and expanded in culture as described previously by direct plating (1). Cartilage explants (1-2 mm thick and 2mm diameter) were obtained from the tibia plateau, placed in 10% fetal calf serum (FCS) containing media (CCM: ICM with 10 ng/ml TGF-β3) for an anoxic 48 h at 37°C and subsequently cultured in serum-free media (ICM) for 24 h. Adenoviral Transduction of hMSCs: Adenoviral (Ad) transduction efficiency was determined using green fluorescent protein (GFP). Adnull and AdBcl-xl was added to cells at a multiplicity of infection (MOI) of 1,000 x 10⁶ for 1.5 hrs at 37°C. Non-transduced hMSCs served as controls. hMSCs viability: Viability of hMSCs alone, Adnull and AdBcl-xl hMSCs was assessed at days 1, 4 and 7 after spin transduction using the Viacount Assay on the GUAVA EasyCyte analyser. Survival of AdBcl-xl Transduced hMSCs: On day 4 post-transduction, 1 or 10 mM hydrogen peroxide (H₂O₂) was added to control hMSCs, Adnull or AdBcl-xl hMSCs for 3h at 37°C. Control wells were treated with serum free media for 3h. Cell viability was determined using the Viacount Assay on the GUAVA EasyCyte analyser. hMSCs Targeted to OA Cartilage Explants: Control, Adnull or AdBcl-xl hMSCs were added to explants 4 days post-transduction at a cell density of 1 x 10⁶ cells/ml for 20 min at 37°C with agitation. Unattached cells were removed by extensive washing and explants were processed for histology immediately or maintained in culture in complete chondrogenic medium (CCM: ICM with 10 ng/ml TGF-β3) for a further 14 days. Cartilage disks incubated without cells were also processed. Formalin-fixed, paraffin-embedded sections were stained with haematoxylin and eosin (H & E), Toluidine blue and immunostained for collagen type I and II.

Results

Cell viability post-transduction and H₂O₂ treatment was assessed. hMSCs in each treatment group were > 80% viable at day 1 and viability increased over the 7 days of culture (Figure 1). H₂O₂ functions as an oxidant and is commonly used as a means of inducing ischemic or anoxic-like cellular changes in cells. AdBcl-xl transduced hMSCs exhibited a significantly enhanced protective effect after 10mM H₂O₂ treatment compared to hMSCs alone (Figure 2). hMSCs alone, Adnull and AdBcl-xl hMSCs adhered to the OA cartilage at 20 min. Bound AdBcl-xl hMSCs survived and started to differentiate to promote more repair of the cartilage surface when exposed to CCM for 14 days (Figure 3, A&D), compared to hMSCs alone (Figure 3). AdBcl-xl hMSCs were demonstrated to increase proteoglycan staining at the surface of the OA explants. Adnull hMSCs had no effect on the OA explants (Figure 3, B&E).

Discussion and Conclusions

Results suggest that adenoviral spin transduction did not affect hMSC viability. AdBcl-xl hMSCs exhibited a protective effect on hMSCs when exposed to 10mM H₂O₂ treatment compared to hMSCs alone. hMSCs, Adnull and AdBcl-xl hMSCs bound to the osteoarthritic cartilage at 20 min. Adherent AdBcl-xl hMSCs underwent a change in morphology and elaborated a more cartilaginous matrix over a 14 day culture period as demonstrated by enhanced proteoglycan deposition compared to control hMSC treated explants. Cartilage repair was also increased with fewer fibrillations and a more even uniform surface evident in these explants treated with AdBcl-xl hMSCs. Adnull hMSCs bound but had no effect on the OA explants. Strategies for stem cell-mediated repair of damaged cartilage as a result of OA may require targeting stem cells to damaged cartilage and their survival at the damage site. In summary, our results suggest that AdBcl-xl transduction does not impair the viability and promotes survival of hMSCs thus making it attractive for the targeted delivery of stem cells to damaged OA cartilage.

References