CHARACTERISATION OF SYNOVIAL AND DERMAL CLONALLY DERIVED MESENCHYMAL STEM CELLS FOR USE IN ARTICULAR CARTILAGE REPAIR

INTRODUCTION
It has long been recognised that injured cartilage does not heal. Partial-thickness defects trigger a minimal and inefficient repair response whilst full-thickness wounds, (which penetrate the underlying bone), induce only a mechanically inadequate fibrocartilagenous scar tissue that degenerates over time. Cell-based approaches to cartilage repair, (e.g. Autologous Chondrocyte Implantation), show potential but are constrained by an inadequate supply of differentiated cartilaginous cells. Recent studies on the biology of cartilage wound margins revealed the existence of a population of ‘cartilage stem cells’ in the superficial surface of growing articular cartilage (1). These presumptive stem cells possess a high proliferative capacity in vitro and an ability to differentiate into mature cartilage. It is uncertain, however, if surface zone progenitor cells constitute the most effective source of mesenchymal stem cells (MSCs) for use in tissue engineering. Early MSCs are known to be distributed throughout adult tissues (2), and many attempts have been made to use these cells to promote orthopaedic repair (3,4,5). This study, therefore, exploits previous methods used to isolate and characterise MSCs from synovium and dermis and assesses the potential benefits for the use in articular cartilage tissue engineering and repair.

MATERIALS AND METHODS
Synovium was dissected from 7-day bovine metacarpal-phalangeal joints and digested overnight in collagenase (0.2% in DMEM + 5% FCS). Dermal tissue was dissected from 7-day bovine limbs and digested in pronase (0.1% in DMEM + 5% FCS) for 1 hour and then transferred (0.04%) in DMEM + 5% FCS) overnight. Differential adhesion to fibronectin (10 mg/ml) was performed at 500 and 1000 cells/ml (1 ml/well, 6 well plates) for synovium and dermis respectively for 20 minutes after which time all non-adherent cells were then removed and grown for 4 days in DMEM + 10% FCS. Colonies >32 cells of synovial and dermal derived MSCs were then cloned and expanded to over 20 Population doublings (PD). Three dimensional pellet cultures were established at 500,000 cell/ml and grown in the presence of medium supporting chondrogenesis, osteogenesis and adipogenesis. Pellets were grown in the presence of the following panel of media for 14 days and fed every 48 hours in order to assess chondrogenic potential: DMEM, 0.5 mg/ml ascorbate-2-phosphate and 1% HEPES buffer (SF); SF medium, 1% ITS Premix (ITS); SF medium, 5mg/ml TGFbeta 1 (TGF); ITS medium, 5 ng/ml TGFbeta1 (ITS/TGF). Pellets were analysed histologically and biochemically for presence of GAGs denoting cartilage formation or osteogenic differentiation, pellets were cultured for 21 days in chemically defined medium (DMEM, 10% FCS, 0.5 mg/ml Ascorbate-2-phosphate, 0.1 uM dexamethasone, 5mM beta-Glycerophosphate, 1% HEPES buffer) and analysed by Von Kossa staining for the presence of bone nodules. For adipogenesis, pellets were cultured over four cycles of adipogenic induction medium (DMEM, 10% FCS, 10 ug/ml insulin, 100 uM indomethacin, 100 uM 3-isobutyl-1-methyl-xanthaninc, 1% HEPES buffer) and analysed by Oil Red O staining for the presence of lipids. Differentiation potential was further assessed by implanting 2 x 10^6 of the MSCs into 2mm diameter full-thickness cartilage wounds excised from 7-day old bovine MCP joints. The defects were cultured for 21 days and grown in the same panel of media used for analysis of pellet cultures for chondrogenesis and assessed histologically for the presence of a GAG rich extracellular matrix (ECM). The plasticity of the MSCs was assessed by injecting PKH26 labeled cells into undifferentiated chick wing buds. After 4 days post-injection, the limbs were cryosectioned and incorporation was visualised using epifluorescence microscopy.

RESULTS
In the case of both synovial and dermal derived MSCs, when treated with a combination of ITS and TGFbeta1 (Fig 1A-D), the overall morphology of the pellets and the presence and distribution of GAGs within the matrix appeared far superior to pellets grown in SF, ITS and TGF media conditions. DMBB assays also revealed that the total GAG content of pellets grown in ITS/TGF for both synovial and dermal MSCs were significantly higher in the majority of cases than pellets cultured in all other media conditions (Fig 1E&F). When grown in the presence of medium supporting osteogenesis and adipogenesis, pellets stained positive for the presence of bone nodules and lipids following Von Kossa and Oil Red O staining respectively. Woundings experiments revealed a similar trend to that of the pellet cultures with optimal conditions for the production of a GAG rich ECM observed in the presence of ITS and TGFbeta1. In ovo plasticity studies revealed that synovial and dermal derived MSCs injected into early undifferentiated chick wing buds are capable of incorporation into connective tissues of the limb including cartilage-associated structures such as the periosteum.

REFERENCES

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