

## A sub-population of human articular cartilage cells display stem cell/progenitor characteristics

[R.Williams](#)<sup>1</sup>; [K.Richardson](#)<sup>1</sup>; [SK.Singhrao](#)<sup>1</sup>; [RE Jones](#)<sup>2</sup>; [DM.Baird](#)<sup>2</sup>; [L Nelson](#)<sup>1</sup> [H.Lewis](#)<sup>3</sup>; [S.Roberts](#)<sup>3</sup>; [J.Dudhia](#)<sup>4</sup>; [IM.Khan](#)<sup>1</sup>; [CW.Archer](#)<sup>1</sup>

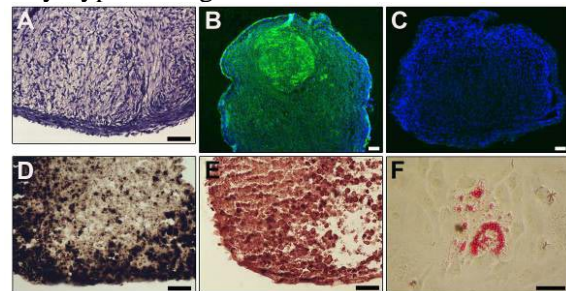
<sup>1</sup>[Connective Tissue Biology Labs](#), Cardiff University, Cardiff, UK. <sup>2</sup>[Cytogenetics Section](#), University Hospital of Wales, Cardiff, UK. <sup>3</sup>[Dept. of Pathology](#), Cardiff University, UK. <sup>4</sup>[Royal Veterinary School](#), London, UK.

**INTRODUCTION:** Tissue engineering procedures are now available which attempt to repair cartilage defects using chondrocytes that have undergone long-term expansion. Chondrocytes in culture, however, often do not retain their chondrogenic phenotype and subsequently repair of the cartilage defect is not maintained long-term. Here we describe the isolation of a population of cells from human articular cartilage that show stem cell/chondroprogenitor characteristics, making this cell population a possible ideal candidate for use in future tissue engineering procedures.

**METHODS:** Human articular cartilage from femoral chondyles (age 10-57 years) was obtained with patient consent and local Health Authority ethical approval. Isolated cells were plated in monolayer or subjected to the fibronectin adhesion assay to obtain clonal cell populations<sup>1</sup>. Clonal 3D pellet cultures were established in chondrogenic or osteogenic differentiation media and adipogenic differentiation was performed in monolayer culture<sup>2</sup>. Histological procedures and immunocytochemistry were undertaken to determine the extent of differentiation. Single length telomere assay (STELA) and quantification of telomerase activities were performed on clonal and full depth cell lines<sup>3,4</sup>. Cytogenetic analysis was used to detect any chromosomal abnormalities in long term cultures.

**RESULTS:** Clonal cell lines obtained using the fibronectin adhesion assay, display a high population doubling, <60. In 3D pellet cultures a chondrogenic phenotype is observed (Fig.1A&B) although collagen type X expression was absent (Fig.1C), indicating that the cells are not becoming hypertrophic. Osteogenic induced pellets display mineralisation (Fig.1D&E) and the adipogenic monolayers show lipid deposition (Fig.1F). Telomere length analysis revealed that both full depth and clonal cell lines undergo telomere

erosion but the telomerase activity of the clonal cell lines was higher than that of the full depth population. Cytogenetic analysis demonstrated that clonal cell lines are able to maintain their karyotype in long term culture.



*Fig. 1: Chondrogenic induced pellets show toluidine blue staining (A), positive collagen type II labelling (B) and the absence of collagen type X (C). Osteogenic induced pellet cultures show von Kossa (D) and alizarin red staining (E). Adipogenic induced monolayers display Oil red O staining (F). Scale bars = 50µm.*

**DISCUSSION & CONCLUSIONS:** Our data suggests that a population of cells displaying stem cell/chondroprogenitor characteristics can be isolated from human articular cartilage using the fibronectin adhesion assay. The isolated chondroprogenitor population can be expanded in culture to over 60 population doublings, displays a strong chondrogenic phenotype, shows multipotency, and retains telomerase activity. At present, it is plausible that this cell population is a stem cell/chondroprogenitor population although further characterisation is required to determine their suitability for use in repairing cartilage defects via tissue engineering procedures.

**REFERENCES:** <sup>1</sup>GP Douthwaite et al., (2004) *J Cell Science* 29:889-97. <sup>2</sup>A Barbero et al., (2003) *Arthritis & Rheumatism* 48:1315-1325. <sup>3</sup>R Capper et al., (2007) *Genes Dev* 21: 2495-2508. <sup>4</sup>IM Khan et al., (2009) *Osteoarthritis & Cartilage* 17:518-528.