

DIFFERENTIATION AND PROLIFERATIVE CAPACITY OF ARTICULAR CARTILAGE PROGENITOR CELLSJ. Bishop¹, M. Smith², S. Webster¹, S. Redman¹, & C. W. Archer¹¹ *Connective Tissue Biology Laboratories, School of Biosciences, Cardiff University, Wales, GB*² *Smith-Nephew, GRC, Heslington Park, York, GB*

INTRODUCTION: In recent years, autologous chondrocyte implantation (ACI) has been increasingly used to repair articular cartilage defects. The number of chondrocytes that can be harvested from a patient for this procedure is limited by biopsy size, and the age of the patient. Previous studies demonstrate that primary chondrocytes as well as having a limited growth potential in culture lose their cartilage-specific phenotype. (1). The dedifferentiated phenotype can be recovered by culturing cells in an environment supporting a spherical morphology. We have identified a population of chondroprogenitor cells in the superficial zone of articular cartilage (2). Here we report on the isolation and extensive expansion of these cells and their ability to redifferentiate when transferred into a 3D environment. The effect of different growth factors on expansion of cloned chondroprogenitor cells was also studied within a 2d monolayer culture to evaluate a procedure to quickly expand a small cell number.

METHODS: *Chondrocyte Isolation:* Surface zones (SZ) chondrocytes were isolated from the metacarpal-phalangeal joints of 7-day-old calves as previously described (2) *Differential Adhesion:* 35mm dishes were coated with 10µg/ml fibronectin in PBS containing 1mM CaCl₂ and 1mM MgCl₂ (PBS+) overnight at 4°C. Fibronectin was removed and SZ chondrocytes plated onto the dishes at 4000 cells/ml in serum free DMEM/F12 and allowed to adhere to the dish for 20 minutes at 37°C. Media and non-adherent cells were removed and the remaining cells cultured for 6 days in DMEM/F12 +10% FCS (DMEM+). *Cloning Chondrocyte Progenitor Cells:* 8 colonies of 32+ cells were identified and isolated using the cloning ring method and cultured in 35mm dishes and cultured in DMEM+. *Expansion in culture:* When cells in the 35mm dish approached confluency (P0), cells were trypsinised and transferred to 25cm² culture flasks (P1). Subsequent growth in 125cm² culture flasks was carried out by continual passage at a ratio of approx 1:6. In addition 56 clones were cultured whereby throughout the expansion phase (P1 and P2) cells were cultured in DMEM+ (1% or 10% FCS) or DMEM+ supplemented with growth factors involved in cartilage metabolism. Factors tested included 5ng/ml FGF-2, 10ng/ml EGF, 10ng/ml PDGFbb, 1ng/ml of TGF-β, and a combination of 5ng/ml FGF-2 and 10ng/ml TGF-β or 10ng/ml IL-1β. *Pellet Culture:* Aliquots of 250,000 cells were resuspended in chondrogenesis media (DMEM supplemented with gentamycin; ITS premix, ascorbate-2-phosphate (100µM) and TGFβ-1 (10ng/ml)). *Histology and immunolabelling:* Pellets were snap frozen in liquid nitrogen and sections of 10µm thickness were cut and stained with Safranin

O/haematoxylin. For immunolabelling, sections were labelled with antibodies to collagen I and II and visualised using appropriate secondary Alexa Fluor 488 conjugated antibodies.

RESULTS: The growth kinetics of the 8 clones were investigated from the primary culture through 17 passages, corresponding to about 45 population doublings. P1 cultures reached confluence in about 2 weeks and an average 18 doublings, a doubling time of less than 24 hours. On replating the clones had slowed their proliferation rate, with a growth rate of about 3-4 days per population doubling. Cultures of cloned progenitor cells comprised a polygonal flattened morphological appearance.

Pellets cultures derived from early passaged chondroprogenitors and redifferentiated in chondrogenic media showed focal areas of glycosaminoglycans (GAG) deposition (Safranin O), were positive for type II collagen labelling and contained small rounded chondrocytes. Interestingly, although pellets derived from P17 stained for GAGs, the labelling for collagen type II was relatively weak. Control pellets derived from full depth isolated chondrocytes contained larger, vacuolated cells, separated by intensely stained matrix. However, pellets derived from full depth cultures at P3 although stained positively with Safranin O had very little collagen type II labelling. Not all the growth factors tested during chondroprogenitor expansion in monolayer induced a significant decrease in the doubling times of exponential growth. However, some growth factors were more effective at reducing doubling times than others. The growth factors, which promoted the highest cell proliferation rates, were FGF-2 and TGFβ1 in combination by significantly reducing the doubling time by 15%.

DISCUSSION & CONCLUSIONS: The data presented in this study indicate that, the population of expanded clones (32+ cells) maintained their ability to proliferate and redifferentiate into a hyaline cartilage like tissue. Expansion of cloned chondroprogenitor cells using FGF-2 & TGFβ1 would also allow faster amplification and to reduce the amount of donor tissue required during repair procedures. It is hoped that the development of a two-phase culture system (monolayer culture followed by pellet culture) using growth factors and chondrogenic media would exploit the chondroprogenitor's already enhanced ability to form cartilage after extensive expansion in culture

REFERENCES. ¹ P.D Benya & Shaffer JD. (1982) Cell 30, 215-24. ² C.W.Archer, et al., (2002) Trams Ors 009.