or ovariectomy (OVX), and administered either vehicle or 30 μg/kg hPTH (1-34aa) by s.c. injection for 5 weeks, n = 10. Serum levels of osteocalcin and CTX-II were measured by specific ELISAs.

Results: When stimulated with PTH, the cultured chondrocytes accumulated intracellular cAMP levels significantly (P < 0.003) in a dose-dependent manner. The maximum concentration of PTH (100 nM) resulted in a 23-35% increase of sulphate by 40% (p = 0.002). The serum level of the bone turnover marker osteocalcin was significantly (p < 0.001) elevated in OVX animals that were PTH treated compared to sham and vehicle treated, while the level of serum cartilage degradation marker CTX-II decreased by 30% (p < 0.01).

Conclusions: The current data strongly suggest that PTH, in addition to osteoblasts and bone turnover, also has direct anabolic effects on chondrocytes and cartilage. We have shown that PTH can not only arrest but also facilitate cartilage generation in both in vitro and in vivo situations. Presented data indicate the potency of PTH and intrigues further investigation of PTH as a potential DMOAD.

**161 NMDA RECEPTOR FUNCTION IN OSTEOARTHRITIC CHONDROCYTES IS DEPENDENT ON β1 INTEGRINS**


**Purpose:** Osteoarthritis (OA) is associated with abnormal loading of articular joints. In normal joints physiological loading maintains structure and function of cartilage and chondrocytes within. This is mediated by a process known as mechanotransduction whereby the mechanical forces are recognized by the resident chondrocytes and transduced into biochemical and molecular responses. In chondrocytes NMDARs are a major mechanoreceptor and activation triggers signaling events and autocrine/paracrine signaling via IL4 which lead to up-regulation of anabolic genes and down-regulation of catabolic activity. In OA however this mechanism is disrupted and mechanically stimulate chondrocytes express pro-inflammatory and catabolic IL1β. The reasons for this are unclear but recent research from our group has raised the possibility that differential involvement of ionotropic glutamate receptors, NMDARs in the mechanotransduction pathway may be important. NMDARs are associated with numerous, although non-neuronal tissues have been shown to express these receptors. In neurons, NMDAR interact with intracellular signaling proteins through the scaffold protein PSD-95. As potential roles for integrins in modifying NMDAR activity have also been shown we have now investigated the relationship between NMDAR signaling in chondrocytes and molecules that are involved in chondrocyte mechanotransduction.

**Methods:** Articular cartilage was removed from OA and chondrocytes released using sequential enzymatic digestion. Chondrocytes were maintained as non-confluent primary monolayer cultures at a concentration of 5 × 10^5 cells/ml. To measure the function of NMDAR cell membrane potentials of OA cells were measured by electrophysiology in resting cells, after treatment (integrin modifying or CD47 antibody (1 μg/ml)) and following stimulation with NMDA. Chondrocytes seeded on cell culture dishes coated with substrates (poly-L-lysine or fibronectin; 10 μg/ml) were used to assess cell membrane potential before and after NMDA treatment. Co-immunoprecipitation of PSD-95 with β1 integrin and CD47 using specific antibodies was analysed by Western blot. Inclusion of OA chondrocytes and anti-CD47 (integrin associated protein) function blocking antibodies inhibited the electrophysiological response to NMDA; antibodies to β3, β5 and anti-α1 integrin antibodies had no effect. Integrin dependency of NMDAR signaling with NMDA treatment was analysed; Chondrocytes adherent to poly-L-lysine (integrin independent attachment) showed no electrophysiological response to NMDA while cells adherent to fibronectin and type II collagen (β1 integrin dependent attachment) showed a membrane depolarization. Co-immunoprecipitation experiments of PSD-95 with β1 integrin and CD47 identified that these molecules are physically linked and therefore may be involved in regulating NMDAR function.

**Conclusions:** We identify that NMDAR function in chondrocyte is linked to β1 integrin and CD47, and that the electrophysiological response to NMDA requires integrin dependent cell-matrix interactions. Linkage of β1 integrins and CD47 to PSD-95, possibly as part of a complex that may contain NMDAR, appears to be important in NMDAR activity.

**162 CHONDROCYTES FROM OSTEOARTHRITIS PATIENTS REVERT TO THEIR ORIGIN PHENOTYPE ONCE GROWN ONTO A HYALURONAN-BASED SCAFFOLD**

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**Purpose:** To evaluate the expression of some specific extracellular matrix molecules in human chondrocytes from healthy and osteoarthritis cartilages freshly isolated and after their growth onto a hyaluronan-based scaffold used in autologous transplantation procedure.

**Methods:** Chondrocytes were isolated from human articular cartilage obtained from the knees of patients with osteoarthritis and from multiorgan donors. First, the cells were expanded in monolayers and then they were seeded on a hyaluronic-acid derivative scaffold. Constructs were analyzed at 0, 3, 7, 14, 21 and 28 days after seeding. Immunohistochemical analysis of type I and II collagen, type I, II, proteoglycans, Sox-9, MMP-1, MMP-13, TIMP-1, cathepsin-B was carried out on freshly isolated cells, on cells grown in monolayer culture and after they were grown onto the scaffold. A Real-Time RT-PCR analysis was performed on the constructs to evaluate the expression of the specific genes at the different experimental times evaluated.

**Results:** Chondrocytes freshly isolated from control and OA patient cartilages expressed the same extracellular matrix molecules even if at different amount. These differences, which were appreciable both at protein and molecular levels, were not evident once the cells were grown onto Hyaff®-11 scaffold. In this experimental culture condition the cells derived from control and OA patients showed a significant increase of collagen type II, Sox-9 and aggrecan and a decrease of collagen type I compared to chondrocytes grown in monolayer. On the other hand, MMPs were downregulated in both the cell types evaluated by the specific action of TIMP-1 which was highly expressed at molecular and protein levels in the two groups.

**Conclusions:** The growth of chondrocytes onto Hyaff®-11 membrane seems to erase the differences between the cells derived from normal and OA cartilages. The hyaluronan-based scaffold is able to recapitulate some embryonic events which allow to the expression of the extracellular matrix specific genes and to the production of the appropriate proteins. This is of particular relevance hypothesizing the use of tissue engineering therapeutic approach also in osteoarthritis patients.

**163 RELATIONSHIP BETWEEN INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR EXPRESSION AND MICROSCOPIC SCORE IN NORMAL EQUINE JOINTS**

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**Purpose:** Osteoarthritis (OA) is a chronic degenerative disease of the articular cartilage which appears to reflect a failure in the attempted repair of the cartilage. Comprehensive data regarding the biological nature of both normal and early OA tissue are lacking. Until such data are available, an accurate comparison of variables between cartilage from different joints and potential therapeutic strategies may be compromised. Insulin-like growth factor-1 (IGF-1) is a potential therapeutic agent in OA due to its ability to have a direct anabolic effect in normal cartilage. In an OA joint, chondrocytes are phenotypically disturbed, and demonstrate altered receptor expression. Disruption in levels of IGF-1 and expression of its receptor (IGF-1R) causes enhanced catabolism of proteoglycans. Therefore the aim of the current study was to investigate IGF-1R expression in macroscopically normal cartilage from grossly normal joints and correlate this with microscopic score, in an attempt to further understand the normal condition of cartilage.

**Methods:** Macroscopically normal cartilage from grossly normal joints was sampled from six sites on the mediolateral meniscal condyle at distances from the transverse ridge (dashed line in figure 1) of