Introduction:
Osteochondrosis dessicans (OCD) is a disorder of unknown etiology where often a fragment of cartilage and subchondral bone separates from the articular surface. The disease is more common in adolescents and young adults, with the knee, elbow and ankle most commonly affected. Many etiologies have been suggested including trauma, ischaemia and additional factors that may predispose patients to the condition (1). Previous studies have shown histological changes in glycosaminoglycan content in OCD cartilage compared to normal cartilage (2). It has also been shown in equine OCD cartilage that there is an excessive degradation of type II collagen compared to normal cartilage (3). The present study was undertaken to examine the gene expression and matrix proteinase activity in human OCD cartilage compared to its normal autologous articular cartilage and human osteoarthritic (OA) cartilage.

Materials and Methods:
Cartilage from OCD patients (18-34 years) was obtained at the time of surgery. Pieces of cartilage were harvested and immediately snap frozen in liquid nitrogen and powdered using a dismembrator. After addition of Tri-reagent (Sigma) to the powdered cartilage, total RNA was isolated using Rneasy minicolumns and reagents (Qiagen, Crawley, UK) according to the manufacturers protocol and RNA eluted in sterile water. Reverse transcription (RT)-PCR was performed using an RNA PCR kit (Perkin-Elmer, Warrington, UK) with oligonucleotide primers corresponding to cDNA sequences for aggrecan, link protein, collagen types I, II and X, decorin, biglycan, versican, aggrecanases (ADAMTS-1, -4 and -5), matrix metalloproteinases (MMP-3 and –13), TIMP-1, -2 and –3, cyclooxygenases (COX-1 and –2), IL-1α and TNFα. Following an initial denaturation step of 1 min at 95°C, amplification consisted of 35 cycles of 1 min at 95°C, 45 sec at the primer annealing temperature, 30 sec at 72°C and a final extension step of 5 min at 72°C. The PCR products were visualised on 3% agarose gels (containing 0.5 µg/ml ethidium bromide) and their nucleotide sequences verified using an ABI 310 Genetic Analyser.

In order to study proteoglycans metabolites, cartilage pieces were extracted in 4M GuHCl for 48 hours at 4°C and analysed using Western blotting with antibodies to aggrecan metabolites (4), collagen metabolites (5) and the small leucine rich proteoglycans (6).

Results and Discussion:
When mRNA expression profiles from OCD cartilage were compared to normal and human OA cartilage, there was an increase in aggrecan, collagen type II nd collagen type X expression. There was no change in expression of link protein or type I collagen when compared to normal cartilage although the expression of these components was decreased in OA cartilage. Expression of the small proteoglycans decorin and biglycan were unchanged in the three cartilage samples, whereas there was loss of expression of versican in the OCD and OA cartilage compared to the normal. The expression of the three ADAMTS enzymes was also different in the three cartilage samples (Figure 1). Neither ADAMTS-1, -4 or -5 was present in the normal cartilage. In contrast, in the OCD cartilage there was expression of both ADAMTS-1 and -4 whereas in the OA cartilage there was expression of ADAMTS-4 and –5. In the case of MMP expression, MMP-3 was decreased and MMP-13 increased in expression in the OCD cartilage compared to both normal and OA samples. The expression of all three TIMP isoforms was increased in the OCD cartilage. Although inflammatory components are not expected in OCD pathology (1), expression of inflammatory mediators such as COX-2, IL-1α and TNFα were all increased in the OCD cartilage when compared to normal cartilage, but expression of these mRNAs was higher in the OA cartilage.

Analysis of proteoglycan fragments in the OCD cartilage by Western blotting showed the presence of a number of aggrecan fragments containing the G1 domain (Figure 2; 7D1). There was also evidence of fragments containing the aggrecan interglobular domain (Figure 2; 6B4) and the C-terminal neoepitope generated by aggrecanase cleavage within the interglobular domain (Figure 2; BC-13). There was also immunoreactivity for biglycan and link protein (Figure 2; αBig and 8A4).

Conclusions:
These results suggest that the phenotypic expression of chondrocytes at the site of the OCD lesion are markedly different from ‘normal’ articular cartilage and also pathological OA cartilage. Interestingly, the expression patterns of matrix proteinases and their natural inhibitors were also markedly different in OCD cartilage again suggesting that there are specific biochemical expression patterns in OCD pathology which may potentially be biomarkers of the disease process. Further studies are necessary to elucidate how the differences in gene expression and matrix protease activity may be involved in the etiology of OCD.

References: