INTRODUCTION
Superficial zone proteoglycan (SZP) is a ~345 kDa proteoglycan that is synthesised by the superficial zone chondrocytes (but not chondrocytes from mid- and deep zones) of bovine articular cartilage and some of the surface lining cells of the synovium [1]. SZP has been demonstrated to be a multifunctional protein that has potential growth promoting and matrix-binding properties associated with the N-terminal domain. Lubricating and cytoprotective properties (provided by mucin-like domains) and matrix binding and aggregating properties are associated with the C-terminal domain. In addition, SZP has been shown to be homologous to the precursor protein of megakaryocyte stimulating factor (MSF) [2]. Immunohistochemical localisation studies reveal that SZP accumulates at the articular surface of cartilage. However, to date, there have been few studies examining expression/localisation of SZP in other musculoskeletal tissues, therefore the present work investigates SZP in both young and mature tendon.

METHODS
Bovine deep digital flexor tendon (DDFT) explants were dissected under sterile conditions from young and mature bovine metacarpophalangeal joints (2-week-old and 18-month-old, respectively). Explants were pre-cultured for 3 days in DMEM and subsequently in serum-free DMEM for 4 days with or without trans-retinoic acid (RA; 10^{-6} M) interleukin-1 (IL-1; 10 ng/ml), tumour necrosis factor-α (TNF; 100 ng/ml), insulin-like growth factor (IGF; 50ng/ml) or transforming growth factor-β (TGF; 2 ng/ml), as described previously [3]. Total RNA was extracted from the tissue explants using a RNeasy kit and reagents (Qiagen), according to the manufacturers protocol. RT-PCR analyses were performed using oligonucleotide primers specific for the C-terminal region of SZP/MSF and the housekeeping gene, GAPDH. Samples of tendon (uncultured) were frozen in OCT compound by immersion in liquid-N₂-cooled isopentane and cryosectioned at 12 µm on to APES-treated slides. Sections were fixed in 4% paraformaldehyde and an SZP-specific monoclonal antibody applied (3-A-4). Visualisation was achieved using biotinylated secondary antibody and avidin-biotin complex reagent followed by DAB/peroxidase reaction (Vector).

RESULTS
RT-PCR analyses revealed that mRNA for SZP was present in control cultures (i.e., constitutively expressed) in both young and mature tendon from compressed and tensional regions (Figure 1). However, treatment with the catabolic agents RA, IL-1 and TNF in young tensional tendon and IL-1 in mature compressed tendon resulted in an apparent reduction in expression of SZP mRNA compared with the control cultures. Immunohistochemical analyses demonstrated that 3-A-4 immunopositive staining was found as a distinct acellular layer that is attached to the tendon surface, predominantly in mature, compressed tendon (Figure 2), similar to previous findings for SZP localisation in articular cartilage [4].

DISCUSSION
This study demonstrates for the first time that tenocytes synthesise and express SZP with expression/localisation occurring differentially with both region and age. Our data also indicate that SZP mRNA may be affected by local and/or systemic catabolic stimuli provided by cytokines. SZP is present as a distinct layer at the tendon surface in mature compressed tendon, which corresponds to the immunolocalisation in articular cartilage. Although young tendon (compressed and tensional regions) and mature tendon (tensional region) also synthesise SZP (demonstrated by the presence of SZP mRNA), it is only detectable at the protein level in mature compressed tendon, suggesting synthesis of much higher levels in this region which increases with age. In a manner complimentary to cartilage, the cells of the superficial zone of tendon may synthesise and maintain a specialised protective matrix at the surface, thus preventing cellular adhesion and facilitating lubrication. In contrast, during pathological events leading to tendon failure including rupture and inflammation (i.e., tendinitis), these physical properties may be lost and thus compromise the integrity and function of the tissue.

REFERENCES

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