

MATRIX DEPOSITION BY TENDON CELLS IN SUSPENSION CULTURE

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INTRODUCTION: Tendons connect muscles to bone, and so must transmit high tensile loads. They consist of longitudinally running parallel bundles of collagen with rows of tendon cells between them. The tendon cells have an intimate relationship with one another and with the collagen bundles¹. Recent *in vitro* studies indicate that the two gap junctions that are important in regulation of tendon cells response to mechanical loading; communication via connexin 32 containing gap junctions upregulates collagen synthesis, whereas via connexin 43 gap junctions does the opposite².

An important aim of tissue engineering of tendons is to replicate such environments *in vitro*. Here we describe the behaviour of tendon cells in a novel 3-dimensional culture system designed to allow cells to establish cell-cell contacts and deposit matrix in the absence of scaffold components and without disturbance by medium changes.

METHODS: Tendon fibroblasts were isolated by sequential protease and collagenase digestion from 50-day-old chicken feet³. Grown to passage 3 in Dulbecco's modified eagle medium (DMEM) with 5% foetal calf serum, 1% antibiotic, 1% L-glutamine at 37°C, 5% CO₂. Cells were suspended at 3x10⁷ cells/ml and 1 ml placed in a DispoDialyzer tube and sealed. This was placed into a 50 ml centrifuge tube with 40 ml DMEM and ascorbate (1mg/ml). After 24 hours, 7, 14 and 21 days the cell aggregates were fixed in 90% methanol (4°C), washed in PBS and rapidly frozen on dry ice in Cryo-M-Bed embedding medium (Cryo-M-Bed Ltd.)

Sections were cut at 10-15 µm, collected on histobond slides and immunolabelled. Sections labelled with polyclonal type I collagen were pretreated with hyaluronidase/chondroitinase. Sections were labelled with monoclonal type II, III and V collagen, actin, vimentin and decorin, connexins 32 and 43, vinculin, Pan cadherin and N-cadherin. Polyclonal antibody binding was detected using FITC conjugated secondary antibody goat anti-rabbit. Monoclonal antibody binding was detected using Alexa488 conjugated goat anti-mouse immunoglobulins. Labelled sections were mounted using Vectashield with propidium iodide. Sections were examined on a Leica Labrolux 12

epifluorescence microscope and photographs taken on a digital camera.

RESULTS: Cells in suspension culture formed elongated aggregates up to 3cm long. Immunolabels showed that at 7 days type I and III collagens were present, predominantly in the periphery. At 14 days the collagens were uniformly distributed throughout the aggregates and showed a degree of organisation. It is also clear from propidium iodide label that the cell nuclei have distinct areas of alignment. The aggregates labelled positively for actin stress fibres, N-cadherin, decorin and connexin 32. Type II collagen and connexin 43 showed no conclusive label.

DISCUSSION & CONCLUSIONS: The suspension cultures clearly show that tendon cells are capable of assembling an extracellular matrix in culture, rather than just releasing their collagen to the media. The structures formed were remarkably similar to tendons in their cell and matrix organisation, with parallel longitudinal rows of tendon cells interspersed with longitudinal collagen fibres. The amount of collagen produced by the cells is affected by time, though quantification must be obtained.

The differences in connexin label may relate to the growth and deposition of matrix. From antisense knockout studies we know that connexin 32 is stimulatory to collagen synthesis and connexin 43 inhibitory². It is important to note the orientation of the cell nuclei corresponding to that of the matrix orientation.

These results suggest that this system is influenced by growth factor combinations and that matrix deposition could be further enhanced and controlled.

REFERENCES: 1. McNeilly et al, (1996). J. Anat. 189, 593-600. 2. Waggett et al, (2001). Trans. Orth. Res. Soc. 26, pp 700. 3. Banes et al, (1988). J. Orthop. Res. 6, 83-94

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