

Effect of tissue thickness and mechanical stimulation on porcine ligament tissue homeostasis

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INTRODUCTION: Acellular natural tissue scaffolds offer a promising solution to tissue engineering of the ACL. Previous work has shown that porcine patella tendons can be decellularised using low concentration SDS¹. Following ultrasound treatment, the acellular tendons retained biomechanical properties and tenocytes were shown to migrate into the scaffold¹. However the matrix became disorganised and cell viability was reduced to 50%¹.

In order to determine the conditions for culture of tissue engineered ACL, the aim of this study was to investigate the effects of dynamic culture and tissue thickness on the maintenance of cell viability and tissue histioarchitecture of porcine tendons.

METHODS: Patella tendons were dissected from hind legs of large white female pigs (35kg) within 1h of humane killing. Firstly, whole tendons (n=3) were cultured under four different conditions: a) clamped static culture b) clamped dynamic culture (1Hz, 6% strain for 4/24h) c) clamped rolled culture and d) unclamped rolled culture (12 rpm) for 6, 48 or 96 hours and 2 weeks. Secondly, tendons were split into individual fascicles or grouped fascicles (~500µm) and cultured under a) clamped static culture b) clamped dynamic culture (1Hz, 6% strain for 4 /24h) or c) unclamped static culture (n=3; all groups) for 96 h and 2 weeks. Prior to harvest (2h) all tissues were injected with hypoxyprobetm-1 (Chemicon). Tissues were harvested, split and samples were cryoembedded, processed for histology, or stained with Live/Dead stain (Molecular Probes) for examination by confocal microscopy. Immunoperoxidase labelling for MMP-1, -2 and -13 and TIMP-1 was carried out on cryostat sections. Histology sections were stained with H&E and labelled with a monoclonal antibody against hypoxyprobetm-1 (Chemicon).

RESULTS: No change in histioarchitecture of any of the whole tendon groups was seen up to 96h. Only those tissues that had been cultured dynamically maintained normal histioarchitecture over the two week period, when clamped static tissues had a looser crimp than clamped dynamic tissues. Unclamped rolled tissues exhibited total

loss of histioarchitecture at two weeks. Tissues which had not been rolled had significantly lower percentages of viable cells (clamped static 70.3±6.91, clamped dynamic 70.0±7.1) compared to rolled tissues (unclamped 99.33±0.65, clamped 99.16±0.8 p<0.05) following two weeks of culture. Hypoxia was observed in non-rolled samples at 48 and 96 hours at depths of >600µm although cell viability was unaffected at 48 hours.

The overall histioarchitecture of dynamically cultured fascicles (individual and grouped) was not affected. The clamped static fascicles showed some relaxation of collagen crimp. The non clamped statically cultured fascicles showed complete breakdown of the extracellular matrix. Hypoxyprobe labelling of fascicles (individual and grouped) showed no evidence of hypoxia and cell viability was maintained in all fascicles for 2 weeks. Labelling for MMP-1, -2 and -13 and TIMP-1 at 2 weeks demonstrated strong positive labelling for MMP-1 and -2 in the non clamped samples. No other labelling was positive.

DISCUSSION & CONCLUSIONS: This study has shown that whilst static tension was able to maintain tissue histioarchitecture in the short term, in order to maintain normal tendon tissue histioarchitecture over two weeks dynamic mechanical stimulation was required. Dynamic culture was able to maintain tendon tissue histioarchitecture even with reduced cell viability. Tissue thickness was a limiting factor for maintenance of cell viability in whole tendon tissue and cell viability could be increased by rolling the tissues, presumably due to an increase in mass transport. Culture of individual tendon fascicles (individual or grouped) prevented hypoxia and cell viability was maintained. The lack of hypoxia in grouped tendon fascicles and the ability to maintain tissue histioarchitecture by dynamic culture *in vitro* is an important step forward in tissue engineering of the ACL.

REFERENCES:¹Ingram *et al* (2004) WBC, Sydney.