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**NOVEL MODELS OF TENDON INJURY AND GAP
JUNCTION MODULATION IN TENDON CELL AND TISSUE
REPAIR**

**A DISSERTATION PRESENTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

*AT MASSEY UNIVERSITY, TURITEA, MANAWATU,
NEW ZEALAND*

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2013

ABSTRACT

Injuries of energy-storing tendons such as the equine superficial digital flexor tendon and the human Achilles tendon are responsible for significant health and financial costs. A good model of acute tendinopathy of energy-storing tendons is required to better understand the changes that occur within the injured tendon and thereby to aid in the development of successful treatment regimens. Connexin43 plays an important role in wound healing and is involved with the spread of cell death signals following injury. Understanding the effect of Connexin43 modulation on tendon injury could improve tendon healing rate and quality.

Models of acute injury were developed in the pelvic limb superficial digital flexor tendon of sheep and adapted for the thoracic limb superficial digital flexor tendon in horses. The sheep injury model was used to investigate the effects of injury on Connexin43 expression and the effect of Connexin43 antisense oligodeoxynucleotides in the peracute (2 to 4 hours) post injury stage. The model in horses was monitored clinically and ultrasonographically and these findings were related to the gross and histological changes at post mortem after 4 days. A surgical model of acute tendon injury was successfully developed and applied to sheep and horse energy-storing tendons. This has potential for modelling tendon injury in horses and humans. Increased Connexin43 levels were measured at the injury site at 2-4 hours post injury. Antisense oligodeoxynucleotides did not significantly reduce Connexin43 levels in the injured tendons in the acute period.

Equine superficial digital flexor tendon-derived fibroblasts derived from different horses exhibit different cell growth rates that may be an indicator of a genetic ability to heal more effectively.

The new models of acute tendon injury may facilitate development of an accurate model of clinical tendon injury in energy storing tendons to improve our knowledge of the problem and our treatments.

ACKNOWLEDGEMENTS

A thesis like any individual's epic journey can only succeed with the support of a team that enrich both the travel and final destination by their involvement. I have been blessed with wonderful people in both my personal and professional lives who have contributed to the completion of this project.

External factors have contributed to changes within my supervisory team however Christine Thomson has been a constant member and deserves great thanks for her advice, patience and support through the numerous challenges. Kathryn Stowell joined the team late on and then became my chief supervisor taking on a postgraduate student with a project involving animals too large to get into the laboratory! Kathryn was the voice of calm never dramatizing the problems, always celebrating the achievements and allowing me to develop my own writing style. Thanks also go to Elwyn Firth and Janet Patterson-Kane who were there at the outset providing advice and help in developing and starting the project.

Many people assisted with various parts of this project, going beyond doing just their jobs to making a real contribution to my study and for that I am very grateful. Colin Green from Auckland University and members of his team (Simon O'Carroll, Xiang Ji and Cameron Johnson) provided invaluable advice and practical help with confocal and gap junction protocols throughout the study. Colin and his wife Paula treated me as part of the family whenever I spent time there and I count them as valued friends as well as esteemed colleagues. Patrick Morel deserves many thanks for all his statistical help. Thanks to my fellow NZ lab rats Sarah Cook, Aaron Gilmour, Sarah Olson and Jaime McDonald, whilst we are now spread far and wide we will always be in the cell culture central family. Also thanks to Sarah Burrows and Dick Wright for getting me started

during my trip to the University of Queensland. Bruce McCann was my 'go to' man for anything mechanical or technical, always being helpful and wearing a smile, he made the days brighter. Bruce alongside the engineering department, in particular Olaf Griewaldt sorted many of my dynamic conundrums. I would like to thank Cameron Knight for examining the sheep histology slides and the rest of the pathology department for all their individual contributions; Mike Hogan, Evelyn Lupton, Matt Perrott, and Keith Thompson. In the equine department, many thanks to Fred Pauwels and Michael Archer for ultrasound image examinations. Also to the nurses, technicians and students for helping with the horse study and to the surgeons, residents and interns for covering as much of the clinical workload as they could so I could write up. I am grateful for the advice and patience of all in MMIC - Dmitry Sokolov, Doug Hopwood, Jianyu Chen – during my confocal and fluorescence microscopy. Much appreciation goes to the office staff especially Lesley England, Gayle McKenna, Debbie Hill and Shirley Morris for help with all things admin.

I am glad my journey has been alongside such special people as Jenny Weston, Linda Laven, Wendi Roe and my fellow post graduate Angels; Jaz Tanner, Charlotte Bolwell and Brielle Rosa. I thank you all for your inspiration, support and friendship.

I have been generously supported by The Equine Trust and a Massey University Vice-Chancellors Doctoral Research Grant. I am very grateful for the funding my studies have received from The Equine Trust, The New Zealand Equine Research Foundation, The Phyllis Irene Grey Trust and the Massey University Postgraduate Research Fund.

I would like to acknowledge the huge contribution my friends here and in the UK have made by always believing in and encouraging me, even if they thought I was mad moving away and studying again! Kathryn Heard, Maxine Igham, Jo Cotton, Stephanie

Sanderson, Sue Dyson, Bruce Bladon, Linda Marshall, Anna Kendall, Phil & Sherryn Bird and Alison & Eddie Fellerhoff. Also, the Super Maori Fullas, other White Ribbon Riders and the people we encountered the length and breadth of New Zealand, I thank for touching my soul and inspiring me to keep moving forward.

My family in the UK - Mum, Nigel, Sally and the Taylor Tribe, Jeanette and the Fulcher family have loved and supported me through all my endeavours. They have given me the strength and desire to push onwards in my career and personal achievements and taught me to be who I am. They are a gift I value and miss every day.

Caitlyn, my stepdaughter has enriched my life as only a child can and has coped amazingly with a step-mum who had so many things going on, and then broke her leg. I am so proud of her and feel blessed that she is part of my world.

Mark is my knight in shining armour and motorcycle leathers. He took me on part way through this project and has cheerfully done all in his power to help me complete. He has become quite an expert on tendon injury (useful when you are a software developer) and thankfully took on the formatting giant for me. You are my hero.

Finally, I dedicate this thesis to Craig Robert Harding (1970-2011) 'a friend who I could be myself with' and without whom this project would never have been completed.

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GLOSSARY

Accessory carpal bone	Carpal bone on the back of the thoracic limb of the horse.
Accessory ligament of the deep digital flexor	Ligament that connects the deep digital flexor tendon to the back of the proximal metacarpus/metatarsus.
Accessory ligament of the superficial digital flexor	Ligament that connects the superficial digital flexor tendon to the back of the carpus/tarsus.
Achilles tendon (human) or Calcaneal tendon	Tendon complex of the soleus and the gastrocnemius tendons that attaches these two muscles to the calcaneus or point of the heel.
Actin stress fibres	Fibres in non-muscle cells involved in cell movement
Acute phase proteins	Proteins whose plasma concentrations alter in response to inflammation
Adhesions	Fibrous bands that form between tissues and organs. A problem in tendon healing as they interfere with the tendon sliding motion.
Aminopropetides	Triple stranded structures at the amino end of procollagen that are cleaved when procollagen becomes collagen.
Angiogenesis	Development of new blood vessels
Anti-sense oligodeoxynucleotides	A complementary sequence to a specific mRNA blocks its expression
Apoptosis	Programmed cell death
Autologous conditioned serum	Serum conditioned to raise the amounts of anti-inflammatory cytokines
Autologous bone marrow	Bone marrow removed from the patient and returned at a distant site
Beta aminopropionitrile fumerate	Interferes with collagen cross-link formation by inhibiting lysyl oxidase
Biomechanical forces	Internal and external forces acting on the body
Bystander region	Region greater than or equal to 400 μm from the injury
Cartilage Oligomeric protein	A glycoprotein component of the extracellular matrix
Collagen	Structural protein family with about 20 members. Collagen I and Collagen III the most common in tendon.
Collagenase	Enzyme that breaks down collagen
Computed tomography	Imaging modality that uses radiographic images to produce cross-sectional slices through the area of interest.
Connective tissue	Supportive tissue that support, connects or separates other types of tissues and organs.
Connexin43	Gap junction protein found in most mammalian cells.
Connexin	Connexins are structurally conserved non-glycosylated integral membrane proteins in vertebrates. They consist of two extracellular facing loops, 4 trans-membrane domains, 1 intracellular

loop and the amino and carboxyl domains on the cytoplasmic side.

Connexon or hemi-channel	Channel in the plasma membrane made up of 6 connexins in a circle around a central pore. Connects the intra and extracellular environments and can dock with another connexon on an adjacent plasma membrane to form a gap junction.
Coronal plane	Also known as frontal plane. Divides body or limb into ventral and dorsal sections.
Corticosteroid	Chemicals that include steroid hormones produced by the adrenal cortex and analogues of these.
Crimp	Planar undulations in the collagen fibres that allow flexibility and energy-storage
Cristae	Multiple inner folds of the mitochondrion that create a large surface area for reactions to occur on.
Cursorial	Adapted specifically to run
Cytokines	Cell signalling molecules
Cytoplasm	Liquid compartment of the cell
Degranulation	Cellular process that releases antimicrobial cytotoxic molecules from secretory vesicles called granules found inside some cells
Desmotomy	Cutting of a ligament
Dimethyl sulphoxide	An organosulphur compound that is a polar solvent.
Dystrophic calcification	Pathological accumulation of calcium salts in a tissue
Eccentric exercise	Lowering phase of an exercise
Elastic modulus	Mathematical description of a substances tendency to be deformed non-permanently when a force is applied to it.
Electrotherapy	Use of small electrical currents into the muscles and tendons as a medical therapy
Endoplasmic reticulum	Network of membrane vesicles involved with protein synthesis
Endothelial cells	Cells lining blood vessels
Energy-storing tendons	Tendons which store elastic energy by reversible stretching of collagen molecules when they are under load. This energy is released with the tendon acting like a spring to aid locomotion.
Epidemiological study	The investigation of the patterns, causes and effects of a condition in a defined population.
Excisional	Removal by surgery
Extra-cellular matrix	Extra-cellular part of animal tissues that provides structural support to the cells in addition to performing tissue specific roles.
Extracorporeal shock wave therapy	Therapy involving the use of high pressure sound waves.
Extrinsic	Extraneous, not part of

<i>Ex vivo</i>	Latin ‘out of the living’ refers to experiments done in or on tissue in an artificial environment not necessarily in culture.
Fibroblast	Most common cells of connective tissue. Responsible for producing the extra-cellular matrix.
Fibro-elastic	Composed of collagen and elastic fibres.
Gap junction	Intercellular junction made up when two hexameric hemichannels/connexons on adjacent plasma membranes dock. Allow molecules of <1kDa to pass through.
Gap junction plaque	Collection of a few to 1000’s of gap junctions in one area of the plasma membrane.
Glucagon	Peptide hormone secreted by the pancreas to raise blood glucose levels
Glyceryltrinitrile	Alternative name for nitroglycerin which is a nitric oxide generator resulting in relaxation
Granulation tissue	The perfused fibrous tissue that replaces the fibrin clot in a wound.
Haematoma	Localised collection of blood outside a blood vessel.
Haemostasis	Process which causes bleeding to stop.
H&E	Haematoxylin and Eosin
Halothane/Isflurane	Inhalational anaesthetic drugs
Hierarchical structure	Arranged in several layers.
Histopathological	Microscopic changes in tissues that indicate the manifestation of disease
Hyalinisation	State of having become hyaline
Hyaluronic acid	Anionic non-sulphated glycosaminoglycan widely distributed in connective tissue.
Hydrotherapy	Part of medicine that uses water for pain relief and treatment. The temperature and pressure of the water are use to stimulating blood circulation, cool the area and provide resistance to movement.
Hygroscopic	Ability of a substance to attract and hold water.
Hypoxic	Reduced oxygen
Immunohistochemistry	The process of detecting antigens(proteins) in tissue sections using the principle of specific antibody-antigen binding.
Incidence	The number of new cases developing a specified condition within a specified period of time.
Insulin like growth factor -I	Or Somatamedin is a protein hormone similar to insulin important in growth.
Intercellular	Between cells
Intracellular	Within a cell

Intracytoplasmic	Within the cytoplasm of the cell
Intralesional	Introduced into or performed within a lesion
Intrinsic	Contained wholly within the organ on which it acts
Ischaemia	Greek – Restricted blood supply
Isometric contraction	Force generation within a muscle without a change in length
<i>In vitro</i>	Latin ‘within the glass’ refers to experiments in culture.
<i>In vivo</i>	Latin ‘within the living’ used to describe experiments involving a whole living organism.
Laminin	Trimeric cross-shaped protein in the base layer of tissues.
Lysyl oxidase	Copper dependent enzyme that cross-links collagen and elastin.
Macrophages	Greek ‘big-eaters’ differentiation of monocytes in tissues responsible for engulfing apoptotic cells and pathogens.
Magnetic resonance imaging	Imaging modality that uses a powerful magnet to align protons within the body in one direction. When the magnet is switched off, the protons return to their normal alignment, releasing energy as they do so. This energy is interpreted by software to create an image.
Matric metalloproteinases	Enzymes that breakdown components of the extra-cellular matrix.
Mesoderm	Middle of the three germ layers in the early embryo
Metacarpal (horse)	Related to the metatarsal bone (cannon) which lies between the carpus and the metacarpophalangeal (fetlock) joints.
Metatarsus	Related to the metacarpal region that lies between the tarsus (hock) and the metatarsophalangeal (fetlock) joints.
Micro-damage	Sub-clinical injury
Mimetic peptides	Proteins that bind to the connexon/hemi-channel and block communication with the extracellular environment. At high concentrations can also uncouple gap junctions.
Mitochondrial	Relating to the mitochondrion, a membrane intra-cellular organelle responsible for energy production.
Morbidity	A diseased state or illness
Mucoid degeneration	Conversion of the cell or tissue substance into a glutinous substance like mucus.
Myofibroblast	Muscle connective tissue cell
Myotendinous Junction	Region of highly folded tissue between the muscle fibres and the tendon.
Necrosis	Greek ‘death’ Cell injury that results in premature cell death
Non-specific gap junction blockers	Substances that block gap junction communication in all types of gap junctions e.g. octanol, carbenoxolone and inhalation anaesthetics. New blood vessel ingrowth

Neovascularisation	
Neutrophils	The most abundant white blood cells in mammals. Important in innate immune responses.
Palmar annular ligament	Fibrous band of tissue around the metacarpophalangeal joint in horses
PBS	Phosphate buffered saline
Plasma membrane	Lipid bilayer membrane around mammalian cells.
Platelet rich plasma	Blood plasma enriched with platelets. The idea being that they contain and release growth factors and cytokines beneficial in healing of bone and soft tissues.
Polymerase chain reaction	Molecular biology technology to amplify a single or a few copies of a piece of DNA across several orders of magnitude to improve detection.
polymorphonuclear cells	Another term for Granulocytes. White blood cells that contain granules in their cytoplasm and have varying shapes of nucleus.
Polysulphated glycosaminoglycans	Semi-synthetic glycosaminoglycan predominantly chondroitin sulphate.
Prevalence	The proportion of a given population found to have a specified condition at a given point in time.
Prostaglandin E1 and E2	Locally acting messenger molecules derived from fatty acids
Proximally	Towards the trunk
Pyknotic	Appearance of irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis
Radiofrequency therapy	Therapy for pain that acts by interruption of nerve conduction on a semi-permanent basis.
Re-epithelialisation	Proliferation of epithelial cells that then migrate across the wound bed to heal the wound.
Regeneration	Restoration of the original tissue or organ to its normal state following injury.
Regional anaesthesia	Local anaesthesia affecting a significant part of the body for example half of a limb.
Risk factor	Variable associated with an increased risk of disease or infection.
mRNA	Messenger ribonucleic acids that are responsible for carrying the code for protein synthesis
RTV	Silastic elastomer used to make stretchable culture dishes
Scarring	Areas of fibrous tissue that replace tissue following injury
Sclerotherapy	Injection of a substance to shrink blood vessels.
Sharpey's fibres	Collagenous fibres that connect the tendon to the collagen in the bone.
Sonogram	A diagnostic image created by echoes of ultrasound waves.

Stem cells	Cells found in all multicellular organisms, which can divide and differentiate into diverse specialized cell types.
Subcutaneous	Under the skin
Superficial digital flexor tendon (equine/ovine)	<p>Tendon of insertion of the superficial digital flexor muscle. In the thoracic limb, the muscle originates on the humerus and caudal radius and inserts on the distal first phalanx and proximal second phalanx. Flexes the carpus and distal limb joints and supports the limb in the stance phase.</p> <p>In the pelvic limb originates on the femur and acts to extend the tarsus and flex the distal limb joints. Forms part of the 'reciprocal apparatus'.</p>
Tenascin-C	Extra-cellular matrix glycoprotein important in development and repair
Tendinitis	Inflammation of tendon
Tendinopathy	Pathology/disease of tendon
Tendinosis	Chronic degeneration of tendon
Tendon	Collagenous structure that transmit tensile force from muscle to bone.
Tendolipomatosis	Accumulation of lipid cells between tendon fibres
Tenoscopy	Endoscopy of tendon sheaths in horses.
Tensile force	Resistance of a material to the force trying to pull it apart.
Transection	Division of a structure by cutting transversely
Transforming growth factor β-I	Polypeptide member of the growth factor beta superfamily of cytokines
Tuber calcaneus	Point of the tarsus where the gastrocnemius attaches
Ultrasonography	Imaging modality that uses the reflection pattern of high frequency sound waves from tissues to create an image.
Vacuoles	Enclosed membrane compartment containing inorganic and organic molecules
Vasoconstriction	Narrowing of blood vessels due to contraction of the muscular wall of the vessel
Vinculum	Bond or tie, in horses it is the attachment of the superficial digital flexor tendon to the digital flexor tendon sheath wall.