Ex-Vivo Organ Culture Study of Ovine Enthesis under Degenerative and Regenerative Conditions

Cassandra Chetty

Supervisor(s): PD Dr. med. Michael Schär, Prof. Dr Benjamin Gantenbein, Dr. med. Slavko Corluka
Institution(s): University Hospital Bern (Inselspital), Department of Orthopaedic Surgery and Traumatology
University of Bern, Department of Biomedical Research (DBMR)
Examiners: PD Dr. med. Michael Schär, Prof. Dr Benjamin Gantenbein

Introduction

A rotator cuff injury affects both younger and older patients; however, the incidence of rotator cuff tears increases with age [1]. The most common rotator cuff tear involves either the partial or complete detachment of the supraspinatus tendon from the humeral head due to injury to the supraspinatus enthesis [2].

The 'Supra-reactor was designed as an ovine enthesis-specific bio-reactor in response to the need for further research into ex-vivo supraspinatus enthesis studies. The aim of this dissertation was to conduct establishing experiments to develop a degenerated ovine enthesis model and a positive treatment/augmentation ovine enthesis model that can be implemented using the current Supra-reactor apparatus.

Both models need to be able to allow for enthesis samples to be cultured for a period of seven days whilst fixed in the mounting apparatus of the Supra-reactor. After the culture period, the enthesis tissue had to show indicators of degeneration or positive treatment in the following assays to validate the degeneration or treatment model: Alamar Blue assay, DMMB assay, Hoechst DNA assay, and Hydroxyproline assay.

Materials and Methods

For the establishing experiment, sixteen ovine enthesis explants were subjected to four different treatment conditions (Genipin treatment, Collagenase Type 1 Treatment, Free-Floating Treatment and 'Healthy' treatment). Free-floating treatment involved culturing the explant in a free-floating condition for seven days. The other treatments involved the explant being mounted into a Supra-reactor chamber with an applied tensile load of five Newtons and then culturing the explant for seven days.

The Free-floating treatment and 'Healthy' treatment involved injecting the explant with phosphate-buffered saline solution. The Collagenase treatment involved injecting Collagenase Type 1 solution into the explant prior to culture to induce a degenerated state. The Genipin treatment involved injecting a solution of the natural collagen cross-linker Genipin into the explant prior to the culture period.

Results

Of the sixteen enthesis explants, only twelve were able to be digested for biochemical analysis. For the degeneration model, some of the degeneration indicators, like increased apoptosis and decrease in total collagen content, were confirmed by the results of the Alamar Assay, DNA Assay and Hydroxyproline Assay.

For the augmentation/positive treatment model, almost all of the Genipin treatment indicators were confirmed to various degrees with the results of each of the assays. These indicators include Genipin's lack of significant effect on total collagen content, Hydroxyproline content, DNA content and GAG content. It also included the indicator of reduced cell metabolic activity and cell viability as the culture time period increases.

Discussion

Prior studies conducted with tendon explants have shown that Collagenase can be used as a means of degeneration to simulate the pathological degenerated state and that Genipin can be used as a means of augmentation to improve the mechanical properties of a degenerated tendon. The degeneration model and positive treatment/augmentation model can be considered valid to a certain extent. However, if a larger sample population is used along with some of the suggested changes to reduce the effect of contamination on the data, then the validity of the models would be increased to a point where it can be used with the applied force elements of the Supra-reactor for different loading cycles and longer cultivation periods.

References

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