Development of a Novel Bioreactor for the Mechanobiological Study of the Rotator Cuff Enthesis

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Introduction

Rotator cuff tear (RCT) is a common injury for individuals over 50 years old, with a prevalence greater than 20% [1]. Once torn, RCTs are treated surgically in most cases. Unfortunately, the surgery has an unsatisfactory failure rate of up to 94% due to poor enthesis healing. *Ex-vivo* models developed in the past investigate the tendon mid-substance but not the rotator cuff as a connected organ, including the enthesis. Consequently, these models do not adequately represent the clinical issue and are unsuitable for translational research. Therefore, a standardized and more complete RCT model is required. We aimed to i) develop and validate a bioreactor for future study of enthesis healing on human samples. Further, ii) perform a preliminary investigation on the influence of mechanical loading on cell viability in isolated entheses from freshly slaughtered sheep.

Materials and Methods

i) For the linear-stage bioreactor, a stepper motor, load-cell, and linear guide were combined and mounted inside a standard incubator for cell culture. A microcontroller did the communication between the load cell and the stepper motor.

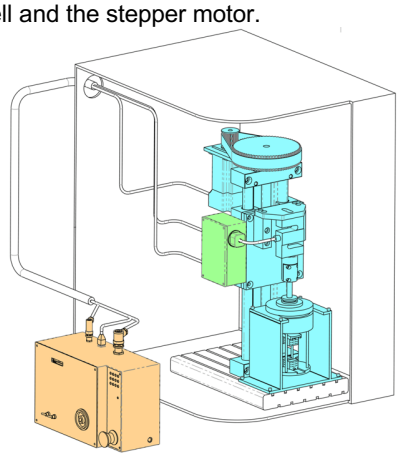

Fig. 1 Scheme of the novel Bioreactor in a standard Cell Culture Incubator with a 5% CO_2 atmosphere and at 37°C.

For the organ culture, sheep infraspinatus entheses (N = 4) obtained freshly from a local were prepared under aseptic conditions and then cultured in high glucose Dulbecco's Modified Eagle Medium containing 5% fetal calf serum in a normoxic environment at 37°C. Samples were then either cultured in an unloaded state (= "free-floating") or under mechanical loading (= "loaded") for up to eleven days. The cell viability was assessed on days 0, 4, and 11 for the free-floating samples and on day 4 for the loaded model.

Results

i) A bioreactor that controls *ex-vivo* enthesis loading based on predefined strain or force values was developed. ii) The free-floating enthesis samples showed a cell viability of 97.6% on day 0, 63.9% on day 4, and 16.5% on day 11. The mechanically stimulated sample showed a cell viability of 74.4% on day 4.

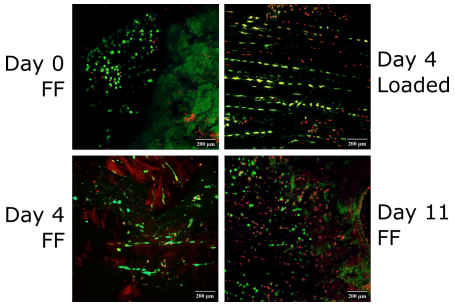

Fig. 2 Representative images of cell viability assessment. The entheses were stained to visualize live cells (green) and dead cells (red) with a confocal fluorescence microscope., FF = Free Floating, Loaded = Mechanically Loaded. 850x850µm per picture

Discussion

i) Most importantly, the newly built bioreactor demonstrated sufficient accuracy and repeatability for research purposes. This makes the bioreactor suitable to be used for mechano-biological studies of entheses. In the future, it will be used to develop a standardized pre-clinical bioreactor model to study enthesis healing. ii) The preliminary results showed a trend that the applied mechanical loading in the *ex-vivo* bioreactor culture of entheses positively influenced cell viability. However, these results need further evaluation in future studies with donor repeats.

References

[1] Wildemann et al., Biological aspects of rotator cuff healing, Muscles ligaments and tendons journal 1(4), 161

Acknowledgments

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