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Experimental Stress Analysis

# Comparison between Mechanical Properties of Fresh and Frozen Specimens of Corio-Epidermal Junction of Equine Hoof

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**Abstract:** The contribution describes an application of a custom software for automatic evaluation of mechanical measurements, namely modulus of elasticity for small as well as large deformations, tissue preconditioning, etc. This software, written in the Python programming language, is applied to mechanical measurements of fresh and frozen samples of corio-epidermal junctions of equine hoof. There were found no significant differences between mechanical properties of frozen and fresh of corio-epidermal junctions.

**Keywords:** Mechanical measurement, Stiffness evaluation, Python

## 1. Introduction

The mechanical properties of soft biological tissues including the corio-epidermal junction of equine hoof are characterized by non-linear response when exposed to the uniaxial tension loading [1-3]. The non-linearity of stress-strain curve is given by very complex structure of the tissue itself. The majority of biological tissues is composed of cells which produce the extracellular matrix including collagen and elastin. The collagen fibers are in the unloaded state highly crimped. At the small deformations the collagen fibers stay crimped and the mechanical reaction of the tissue is given only by soft elastin fibers. This corresponds to the linear relation between stress and strain, so called 'toe' region, characterized by low stiffness. As the loading grows the tissue begins to elongate and the crimped collagen fibers start to straighten, the stress-strain curve gets in the so called 'heel' region which is characterized by tissue stiffening connected with partial participation of collagen fibers in the tissue response. With higher loading the collagen fibers become totally straight and the tissue response is fully in competence of stiff collagen fibers. The tissue stiffness in this 'linear' region is higher than in the previous regions. With further loading the tissue components start to failure and surpassing the ultimate strength leads to the total tissue rupture.

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To make easier the evaluation of mechanical properties of tissues, especially soft biological tissues, we develop the program elfpy (Experimental lab fits in Python [4]) written in the programming language Python. The basic functions cover the reading and loading of input data, evaluation of uniaxial mechanical tests, the graphical interpretation of results (stress-strain curves with moduli of elasticity) and the saving of results. We employ standard program packages such as numpy, matplotlib and ipython.

Key features of elfpy are:

- evaluation of one or more mechanical measurements, either independent or mutually dependent;
- automatic determination or interactive definition of the regions of small and large deformations;
- automatic determination of the ultimate value of stress and strain finished by tissue rupture;
- evaluation of cyclic loading.

This software was used for evaluation of mechanical measurements of corio-epidermal junctions of equine hoof.

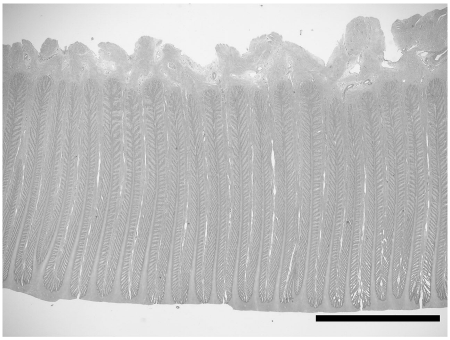

**Fig. 1.** The leaf-like primary lamellae of corio-epidermal junction. Bar = 2000 μm.

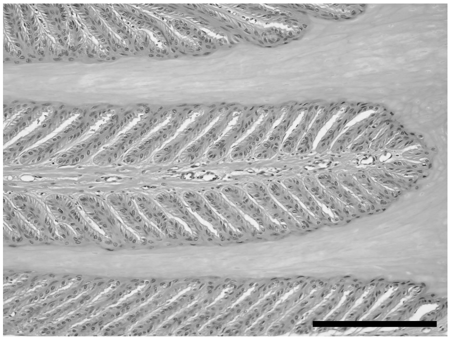

**Fig. 2.** The secondary epidermal lamellae covering the primary lamellae. Bar = 200 μm.

The weight of an adult horse is about 400 kg. This weight is settled on four equine feet, namely on the flexible suspensory apparatus of hoof of the equine foot [5]. The suspensory apparatus connecting the hoof and the distal phalanx is formed by corio-epidermal junction created by 500-600 parallel, leaf-like primary lamellae (Fig. 1). The primary lamellae are covered by about 150-200 secondary lamellae along the length of each of the primary lamellae (Fig. 2). The secondary lamellae are tightly connected with their basement membrane forming the interface of the lamellar epidermis and dermis. Precisely this tight connection between epidermal lamellae, firmly attached to the periostium of the digital phalanx, and dermal lamellae rising from the equine hoof enable supporting the 400 kg of the horse.

From this point of view it is very interesting to investigate the mechanical properties and the structure of this amazing tight junction.

When we measure a biological tissue we should ask how to obtain and measure the fresh specimens of the tissue as soon as possible to prevent the possible degradation of the tissue. Because the study of the structure and the mechanical properties of corio-epidermal junction arises from cooperation of distant workplaces and the preparation of the measured specimens is highly demanding we would like to stop the degradation of the tissue by freezing the tissue specimens. There are no studies describing the influence of freezing on mechanical properties of this kind of tissue. Therefore we compared the mechanical properties of fresh and frozen specimens taken from one animal.

## 2. Material and Methods

### *2.1. Mechanical measurement*

The mechanical properties of fresh and frozen tissue specimens were determined using uniaxial tensile test. The tissue specimens (about 20 mm in length, 10 mm in thickness and 6 mm in width) taken from the front (F), quarters (lateral parts of the foot, Q) and heel (H) parts were cut by saw from the front feet of a foal. Individual specimens, namely the bone of the distal phalanx and the wall, were clamped into the pneumatic grips of a traction machine (Zwick/Roell GmbH & Co, Ulm, Germany) so that only the corio-epidermal junction was exposed to mechanical loading. Following the previous study [6] the specimens were firstly preconditioned, i.e. exposed to a cyclic loading. The cyclic loading involved 50 cycles, one cycle consisted of a linearly growing elongation up to strain 18.00% and a symmetrical shortening back to the initial length of each specimen. The loading velocity was 500 mm/min for both parts of the cycle. This elongation rate corresponds to the velocity that is produced by equine gallop [7].

After this preconditioning, the tensile test with a linearly growing elongation up to tissue rupture was applied. The stress-strain curves were recorded. The Young's modulus of elasticity for each cycle (cyclic loading), and the Young's modulus of elasticity of toe and linear regions and the ultimate stress and strain for tension (tensile test) were all determined using elfpy.

### *2.2. Statistical analysis*

Statistica base 9 (Statsoft, Inc., Tulsa, OK, USA) was used for data processing. The normality of the data was tested with the Shapiro-Wilk W test. The differences between mechanical parameters of frozen and fresh specimens were evaluated using the Mann-Whitney U Test.

## 3. Results

The cyclic loading revealed the hysteresis of the tissue response with increasing number of cycles, which is characteristic for biological materials. In other words, a lower force was necessary to reach the same specimen extension with increasing number of cycles for both kinds of the tissue (fresh and frozen). The maximal stresses of the hysteresis curves decreased and the tissue tended towards a steady, preconditioned, state of the tissue (about 10 cycles for the both kinds of tissues). It was evident that the values for the Young's moduli of elasticity decreased with increasing number of cycles in all cases (Fig. 3).

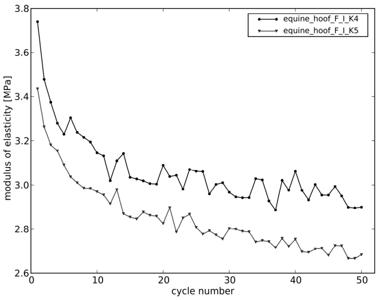


**Fig. 3.** The example of results of cyclic loading for front specimens (F). Young's moduli of elasticity decreased with increasing number of cycles. K4 – fresh specimens. K5 – frozen specimens.

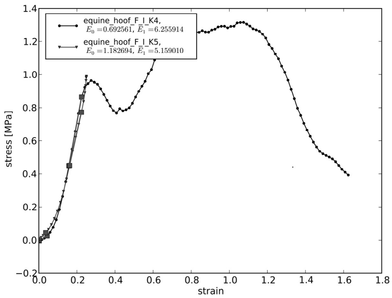


**Fig. 4.** The example of results of linearly growing loading up to rupture for front specimens (F). $E_{toe}$ and $E_{linear}$ - Young's moduli for toe and linear regions of elasticity, respectively. K4 – fresh specimens. K5 – frozen specimens.

Mechanical results further showed that frozen and fresh samples were characterized by a non-linear response when subjected to the linearly growing load. The toe, heel, and linear regions were significantly visible (Fig. 4). The toe and linear regions were approximated using a linear regression and the Young's moduli of elasticity were determined. The resultant values are summarized in Table 1.

## 4. Discussion

The cyclic mechanical loading showed hysteresis of corio-epidermal junction in frozen as well as fresh samples characterized by decreasing of Young's moduli of elasticity of the tissues with increasing number of cycles.

The tissue subjected to the uniaxial linearly growing loading exhibited non-linear response in both cases (frozen and fresh) characteristic for soft biological tissues. The determined values of Young's moduli of elasticity showed a large stiffening of corio-epidermal junction connected with an increased Young's modulus for large deformations with respect to Young's modulus for small deformations for both cases of tissue. Using the Mann-Whitney U Test there were no significant differences between values obtained for fresh and frozen samples of CEJ for small ($p = 0.19$) as well as large deformations ($p = 0.37$).

**Table 1. The resultant values of Young's moduli of elasticity ($E_{toe}$ - the toe region and $E_{linear}$ - the linear region) of samples stored as frozen (K5) as well as of the fresh (K4). F-front, Q-quarter, H-heel, I- upper part, II - lower part, R-right side, L -left side**

| sample | $E_{toe}$ (MPa) | $E_{linear}$ (MPa) |
|---|---|---|
| equine hoof QR II K4 | 0.12 | 8.90 |
| equine hoof QL I K4 | 0.45 | 2.47 |
| equine hoof HR I K4 | 0.19 | 1.88 |
| equine hoof F I K4 | 0.69 | 6.25 |
| equine hoof HL I K4 | 0.22 | 1.24 |
| equine hoof QR I K4 | 0.12 | 7.71 |
| equine hoof QL II K4 | 0.10 | 5.94 |
| equine hoof F II K4 | 0.35 | 13.25 |
| equine hoof QR I K5 | 1.12 | 7.25 |
| equine hoof HL I K5 | 0.32 | 6.74 |
| equine hoof QL I K5 | 1.21 | 6.56 |
| equine hoof HR I K5 | 0.11 | 7.40 |
| equine hoof QL II K5 | 0.13 | 6.21 |
| equine hoof QR II K5 | 0.54 | 8.35 |
| equine hoof F II K5 | 0.23 | 14.03 |
| equine hoof F I K5 | 1.18 | 5.16 |

## 5. Conclusion

We have found no significant differences between mechanical properties of frozen and fresh of corio-epidermal junction. The frozen samples can be so used for further investigation of this kind of tissue.

### Acknowledgements

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