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Comparison of Optical and Biomechanical Properties of Native and Artificial Equine Joint Cartilage under Load using NIR Spectroscopy

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Abstract

The gold standard for functional analyses of joint cartilage tissue is biomechanical indentation. There, parameters like modulus of elasticity or modulus of relaxation are evaluated. A fast and non-destructive method to characterize cartilage properties is near infrared spectroscopy (NIRS). It is sensitive to the molecular structure and morphology of the tissue. A combination of NIRS and biomechanical indentation offers a new possibility to study functional behaviour of tissue. Especially for tissue engineering this non destructive method has the potential to improve the process monitoring and quality control. For osteoarthritis research and diagnostics the functional characterisation of cartilage tissue can be improved. In order to demonstrate the prospects of the method, the optical and biomechanical behaviour of native equine joint cartilage with and without pathological changes and tissue engineered equine joint cartilage will be compared in this paper.

1 Introduction

Near infrared spectroscopy (NIRS) is a fast and non-destructive method to characterize cartilage properties. It is sensitive to the molecular structure and morphology of the tissue [1] and can be used to evaluate cartilage lesions during arthroscopy [2]. A combination of this optical method with biomechanical indentation offers a new possibility to study functional behaviour of tissue. NIRS of loaded cartilage tissue reveals processes on molecular scale like the water binding capacity under load which are non-accessible to standard tissue analysis [3]. The method can be applied to native as well as artificial cartilage as will be shown.

2 Methods

DTK: As examples for artificial tissue we used two three-dimensional scaffold-free tissue engineered cartilage constructs DTK 1 and DTK 2 like one shown in Image 1a. The tissue was cultivated at the fzmb by a method described in [4] using chondrocytes of adult equine donors. The constructs had a diameter of about 7.0 mm and a height of about 1.3 - 1.7 mm.

The samples of native cartilage were extracted postmortem from two knees of two adult horses. The bone cartilage cylinders shown in Image 1b were extracted using a trepan drill. The cylinders had a diameter of 10 mm with a cartilage thickness of about 2.0 - 2.5 mm. In order to avoid drying and to minimize post mortal changes of the tissue, the joints were wetted during excavation and the samples were stored in physiological saline solution until measured.

NKP: Both knees did not show cartilage defects in the patella region. Here, the samples NKP 1 and NKP 2 were extracted in the localization shown in Image 1c. The samples NKP 1 and NKP 2 represent healthy native equine joint cartilage (ICRS 0).

NKF: At the Condylus medialis femoris, both knees showed signs of cartilage degeneration like tissue softening, fissures and discoloring. The samples NKF 1 and NKF 2, extracted in the localization shown in Image 1c, represent equine joint cartilage with initial pathological changes (ICRS 2).

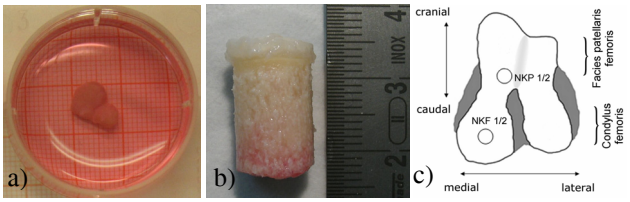

Image 1 analyzed cartilage samples: **a)** artificial tissue DTK **b)** native tissue **c)** sample localisation NKF / NKP

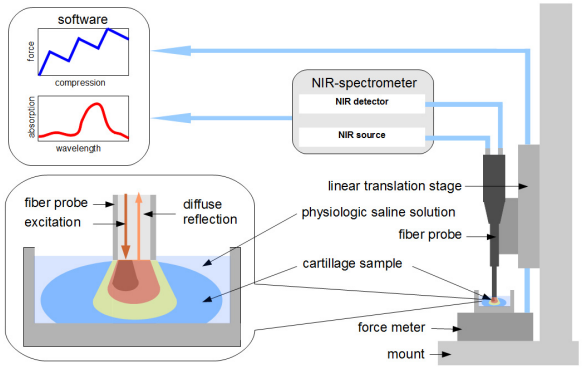

Image 2 instrumental setup for NIRS in combination with biomechanical indentation

The instrumental setup is shown in Image 2. Main component is a NIRS System (arthrospec, Jena) specially designed to assist arthroscopic diagnosis. The NIRS System is synchronized with a linear stage (Thorlabs, Dachau) to drive a 2 mm fiber probe. A force sensor (ME-Messsysteme, Henningsdorf) is used to measure the mechanical load caused by indentation.

For measurement the samples were mounted in a vessel filled with physiological saline solution shortly after excavation. The fiber optic probe was placed perpendicular to the tissue surface. With a feed rate of v = 0.5 mm/s the fiber probe was driven to compress the tissue by Δs = 0.1 mm. During indentation, the applied force F(t) is measured with a sampling rate of 32 Hz. For spectral analysis reflection spectra within the spectral range of 950-1650 nm were recorded with a sampling rate of 9 Hz. From the recorded reflection spectra the absorption spectra A(λ,t) and the change of absorption ΔA(λ,t) were calculated in reference to a PTFE reflection standard. For the graphical visualisation the signal noise ration of the recorded data was improved by data pre-processing.

2 Results

The feed of the fiber probe and the resulting tissue compression is shown in Image 3. The compression load F(t) during indentation is shown in Image 4.

During increasing compression the applied force increases linearly and reaches a maximum F_c by t = 0.2 s for all samples. During constant compression the tissue relaxes and the compression load decreases exponentially to F_R by t = 60 s. For the artificial samples the relaxation process has subsided within the experimental time. For the native samples the relaxation still proceeds. With $F_c(NKP) > F_c(NKF) > F_c(DTK)$ and $F_R(NKP) \approx F_R(NKF) > F_R(DTK)$ the samples NKP shows the highest stiffness and highest relaxation ratio.

The absorption spectra of the uncompressed tissue by t = 0 s is shown in Image 5. All samples show a typical spectral characteristic of cartilage with an absorption maximum at 1453 nm, a local maximum at 1175 nm and an increased absorption at 950 -1000 nm.

During the compression phase (0 s < t < 0.2 s), the absorption decreases within the whole spectral range for all samples. Within the phase of constant compression (0.2 s < t < 60 s), the change of absorption varies considerably between the samples. This is shown exemplarily at two wavelengths 950 nm and 1453 nm in Image 6 and Image 7.

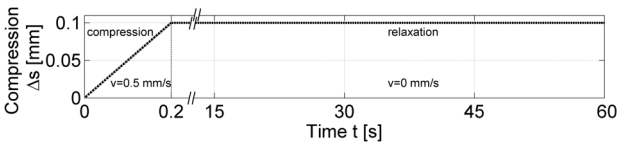

Image 3 tissue compression during indentation

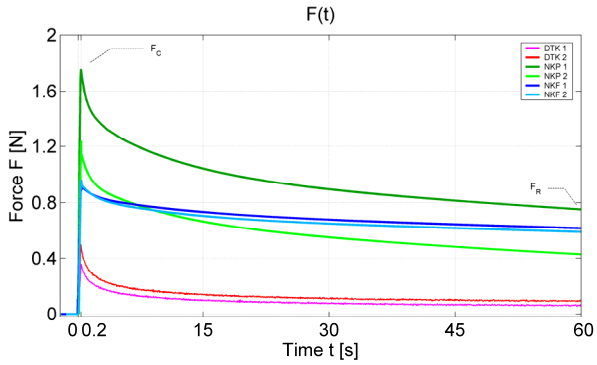

Image 4 compression load during indentation

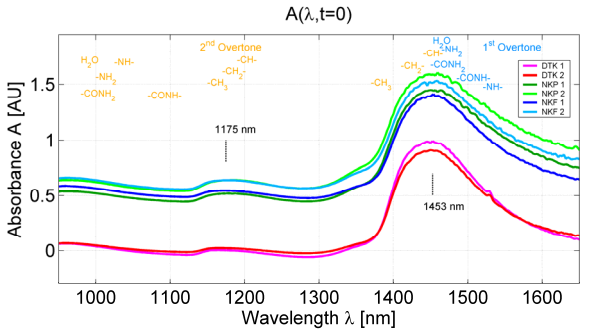

Image 5 absorption spectra of uncompressed tissue

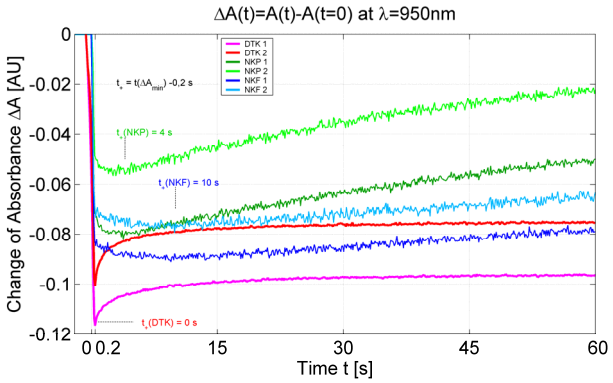

Image 6 change of absorption at 950 nm caused by indentation

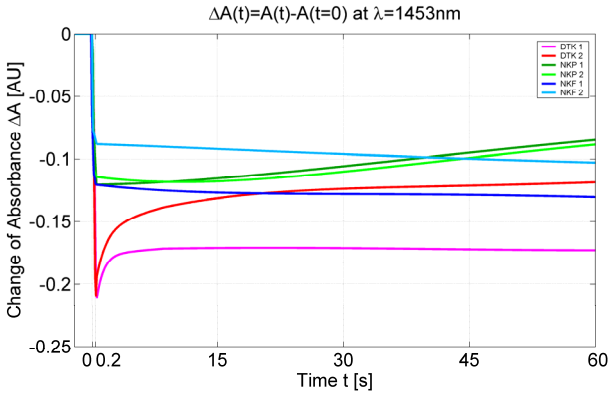

Image 7 change of absorption at 1453 nm caused by indentation

During relaxation, the absorption ΔA_{R950} increases for all samples at 950 nm. This increase is delayed by $t_+(NKP) = 4$ s and $t_+(NKF) = 10$ s for the native cartilage. For artificial cartilage the absorption increase starts without delay $t_+(DTK) = 0$ s immediately after compression.
At the absorption maximum of 1453 nm, the change of absorption ΔA_{R1453} of the samples DTK 1 / 2 and NKP 1 / 2 is positive whereas the change of absorption of the NKF samples is negative.
The tissue parameters discussed are summarised in Table 1.

Table 1 tissue parameters

	DTK1	DTK2	NKP1	NKP2	NKF1	NKF2
ICRS	-	-	0	0	2	2
F_C [N]	0.36	0.5	1.74	1.24	0.91	0.96
F_R [N]	0.07	0.1	0.75	0.62	0.62	0.59
t_+ [s]	0	0	4	4	10	10
ΔA_{R950} [AU]	0.02	0.025	0.032	0.028	0.006	0.006
ΔA_{R1453} [AU]	0.03	0.092	0.036	0.025	-0.01	-0.015
Höhe [mm]	1.3	1.7	2.0	2.2	2.5	2.4

3 Conclusion

The measured force response with a linear increase during compression and an exponential decrease during relaxation for all samples is typical for cartilage tissue. The differences in absolute values represent the variation in stiffness and relaxation capability of the samples.
Also the absorption spectra of the uncompressed tissue are typical for cartilage. The absorption maximum at 1453 nm corresponds to the first overtone region of H_2O, $-NH_2$, -CONH- and $-CONH_2$ groups. The local maximum at 1175 nm corresponds to the second overtone region of $-CH_2$ groups and the increased absorption at 950 -1000 nm corresponds to the second overtone region of H_2O, $-NH_2$ and $-CONH_2$ groups [5]. The absorption offset between native and artificial tissue can be explained by the different supporting structure which was bone for the native samples and PTFE for the artificial samples.
According to the biphasic model the biomechanical behavior of joint cartilage can be explained by the interaction of the elastic collagen structure and the interstitial fluid. During compression, the density of the collagen structure increases and the interstitial fluid is squeezed out [6]. That leads to reduced water content, the main compound of cartilage and thus the main absorber within the observed spectral region. Subsequently NIR absorption decreases during compression as shown in Image 6 and Image 7. This process of water displacement during compression is observed for all samples. Especially dominant is this effect at 1453 nm, the first overtone region of water.
During relaxation the displacement of interstitial fluid continues until equilibrium of hydrostatical and mechanical pressure is reached. Therefore, the decrease of absorption should continue until relaxation has subsided. At 1453 nm, the native samples without pathological changes NKP show this behavior, whereas the absorption of the samples NKF and DTK increases. This can not be explained by the process of water displacement and must be the result of superimposed processes caused by compression which lead to an increase of absorption. At 950 nm, the increase of absorption is delayed with characteristic delay time for the samples DTK, NKF and NKP.

The classic biomechanical indentation allows the evaluation of the mechanical function of joint cartilage tissue in terms of elasticity modulus or relaxation modulus. Additionally to these established parameters, the NIRS of loaded cartilage gives an access to molecular processes influencing the functional behaviour of cartilage.
Native cartilage with and without pathological changes and tissue engineered cartilage reveal different spectral characteristics under load even if the general mechanic response is comparable. The specific spectral changes can be evaluated and used to improve the functional characterisation for native as well as artificial tissue. Especially for tissue engineering this non destructive method has the potential to improve process monitoring and quality control. For osteoarthritis research and diagnostics the method can help to advance the functional characterisation of cartilage tissue.

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