

# AN INTEGRATED FRAMEWORK FOR EVALUATING MECHANICAL PROPERTIES AND STRUCTURE OF ARTICULAR CARTILAGE

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## Introduction

Articular cartilage is a highly specialized connective tissue covering diarthrodial joints and, thus, playing a key role in supporting locomotion. Non-invasive approach, providing indirect information on the cartilage mechanical properties would be a potential method in early detecting tissue disorders, and evaluating new tissue engineering approaches and therapeutic treatments. Regarding cartilage mechanics, indentation represents an experimental method capable of reaching a high degree of accuracy through specifically designed testing protocols [1]. Concerning their biological structure, single-sided low-field nuclear magnetic resonance (NMR) method recently demonstrated its potential to study both soft and mineralized tissues [ref]. Accordingly, the study aimed at proposing an integrated, non-destructive framework capable of quantitatively evaluating cartilage mechanics and structure, following the main hypothesis that tissue characteristics change across its thickness.

## Methods

Osteochondral specimens ( $N = 18$ ) were cored from bovine knees. Cartilage thickness was estimated through a microCT apparatus (SkyScan 1072). Aiming to determine cartilage viscoelastic response, specimens were tested – five times each – by a multiaxial mechanical tester (Biomomentum), equipped with 6-mm spherical indenter. A specific testing protocol was developed [ref], inducing a nominal deformation of 15% of tissue thickness, equal to the averaged one developed during locomotion; after reaching the imposed deformation, a stress-relaxation of 300 s allowed to investigate tissue viscosity [2]. Hayes model [3] was used to fit the cartilage elastic response, determining tissue instantaneous ( $E_0$ ) and equilibrium ( $E_{eq}$ ) modulus. The stretched exponential model [4] was selected for describing the stress-relaxation response, estimating tissue time constant ( $\tau$ ) and stretching parameter ( $\beta$ ) starting from the measured maximum load ( $S_0$ ).

Specimens were then analyzed by a NMR single-sided device (MOUSE PM10, Magritek, NZ). CPMG, Saturation Recovery, Stimulated Spin Echo, and build-up Double-Quantum-like pulse sequences were performed in a unique procedure to determine  $T_2$ ,  $T_1$ ,  $D$ , and a parameter,  $\alpha$ , related to  $^1\text{H}$  solid-liquid ratio. Data analysis – cartilage thickness, mechanical properties, and NMR parameters – was performed by custom-made codes (MATLAB 2022b, MathWorks).

## Results and Discussion

The testing procedure enabled a sound measure of the cartilage viscoelastic parameters, i.e., mean percentage coefficient of variation (CoV%) of 6.1%, 7.8%, 4.3%, 3.5%, and 1.1%, for  $E_0$ ,  $E_{eq}$ ,  $S_0$ ,  $\tau$ , and  $\beta$ , respectively. NMR approach allowed to estimate the thickness of the cartilage (NMR vs microCT agreement:  $r = 0.97$ , Fig.1) and to distinguish its three sub-layers (superficial, middle, and deep). Significant discriminations ( $p < 0.05$ ) among layers were highlighted by all NMR parameters supporting single-sided NMR as a sensitive method to detect cartilage structural changes – i.e., water confinement, proteoglycan and collagen organization.

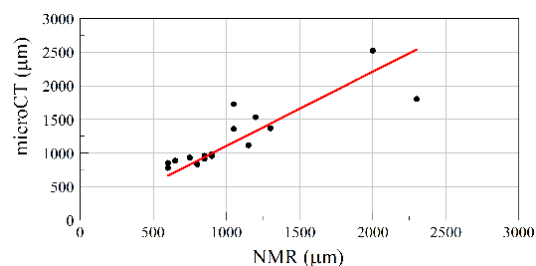


Figure 1. Cartilage thickness evaluation: microCT versus single-sided NMR profiling.

Moreover, preliminary results suggest that cartilage mechanical stress-relaxation is related to NMR relaxation times ( $T_2$  and  $T_1$ ), diffusion coefficient  $D$ , and  $\alpha$  parameter.

## Conclusions

The results support the use of a combined indentation-NMR approach to investigate the main features of cartilage tissue. Future developments will deepen first the relation between tissue response and NMR-derived parameters. Moreover, the proposed pipeline will be applied to both healthy and pathological tissues, to prove the feasibility of the approach in detecting changes in cartilage homeostasis throughout degeneration stages.

## References

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