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Early impairment of cartilage poroelastic properties in an animal model of ACL tear.

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Abstract

Background.

In more than 50% of cases, anterior cruciate ligament (ACL) lesions lead to post-traumatic osteoarthritis. Ligament reconstruction stabilizes the joint, but the tear seems to impair the poroelasticity of the cartilage: synovial membrane fluid inflammation is observed 3 weeks after tearing. There have been some descriptions of visible cartilage changes, but poroelasticity has never been analyzed at this early stage. The present animal study aimed to determine 1) whether cartilage showed early poroelastic deterioration after ACL tear;

2) whether impairment correlated with macroscopic changes; and 3) whether cartilage poroelasticity deteriorated over time.

Hypothesis.

In the days following trauma, cartilage poroelasticity is greatly impaired, without macroscopically visible change.

Material and methods.

ACL tear was surgically induced in 18 New-Zealand rabbits. Cartilage poroelasticity was assessed on indentation-relaxation test in 3 groups: “early”, at 2 weeks postoperatively (n=6), “mid-early” at 6 weeks (n=6) and in a non-operated control group (“non-op”). Macroscopic changes were scored in the same groups.

Results.

Poroelastic impairment was greatest at the early time-point (2 weeks). Permeability ranged from a mean $0.08 \pm 0.05 \times 10^{-15} \text{ m}^4/\text{Ns}$ (range, 0.028-0.17) in the “non-op” group to $1.03 \pm 0.60 \times 10^{-15} \text{ m}^4/\text{Ns}$ (range, 0.24-2.15) in the “early” group ($p=0.007$). Shear modulus ranged from $0.53 \pm 0.11 \text{ MPa}$ (range, 0.36-0.66) to $0.23 \pm 0.10 \text{ MPa}$ (range, 0.12-0.43), respectively ($p=0.013$). Macroscopic deterioration, on the other hand, differed significantly only between the “mid-early” and the “non-op” groups: $p=0.011$ for cartilage deterioration and $p=0.008$ for osteophyte formation. At the “mid-early” time point, poroelastic deterioration was less marked, with $0.33 \pm 0.33 \times 10^{-15} \text{ m}^4/\text{Ns}$ permeability (range, 0.06-1.06) and shear modulus $0.30 \pm 0.10 \text{ MPa}$ (range, 0.13-0.41: respectively $p=0.039$ and $p=0.023$ compared to the “non-op” group.

Discussion.

The severe rapid deterioration in poroelasticity following ACL tear in an animal model, as notably seen in increased permeability, corresponds to changes in cartilage microstructure, with easier outflow of interstitial fluid. This mechanical degradation may underlie onset of microcracks within the cartilage, leading to physiological loading that the cartilage by its nature is unable to repair. Further investigations are needed to correlate these experimental data with clinical findings.

Level of evidence: III; comparative study with control group

Key-words: cartilage poroelasticity, tear, ACL, early deterioration, post-traumatic osteoarthritis

1 Introduction

Anterior cruciate ligament (ACL) tear mainly affects young subjects. The current literature reports post-traumatic osteoarthritis of the knee in more than 50% of case 15 years post-trauma, with progressive cartilage degradation [1]. Ligament reconstruction restores stability [2–4], but is less relevant to preventing long-term osteoarthritis [5]. This is a non-inflammatory pathology, but it is now widely agreed that any joint trauma induces a cascade of biological processes [6] directly impacting synovial membrane inflammation and synovial fluid quality [7,8]. Treatment therefore now focusses on early intervention to modify the membrane, such as hydrogel or glucocorticosteroid injection [9]. Joint cartilage is a complex biphasic material comprising a solid matrix and interstitial fluid [10]. Its microstructure enables it to support mechanical loading and to play a shock-absorbing and lubricating function. It is avascular, with only very slight regrowth capability [11]. Its mechanical behavior is modeled on regime-dependent models relating to the interaction between its solid and liquid phases. The most widely used is the poroelastic model, describing the migration of the interstitial fluid, ensuring chondrocyte nutrition by diffusion, within the solid phase, taking account of the viscosity of the matrix. The two most significant parameters are permeability (K: amenability to fluid flow), and shear modulus (G) in response to friction shear-stress [12].

Four stages are distinguished in post-traumatic osteoarthritis[13]. Within the earlier stages, “early” (up to 3 weeks post-trauma) is distinguished from “mid-early” (4-7 weeks), while later stages comprise “mid-late” (8-11 weeks) and “late” (>12 weeks). “Mid-early” studies report changes in morphology (surface fibrillation), biochemistry (glycosaminoglycan loss) and mechanics (viscoelastic with reduced elastic modulus at equilibrium, and poroelastic, with increased permeability and reduced shear modulus). However, to our knowledge, there are no data for poroelasticity in the “early” stage.

The aim of the present comparative animal study with control group was to determine: 1) whether cartilage showed early poroelastic deterioration after ACL tear; 2) whether impairment correlated with macroscopic changes; and 3) whether cartilage poroelasticity deteriorated over time. The study hypothesis was that in the days following trauma, cartilage poroelasticity is greatly impaired, without macroscopically visible change.

2 Material and methods

2.1 Animal model

This experimental study was conducted in 18 healthy male adult White New-Zealand rabbits (Charles River Laboratories, L'Arbresle, France), with a mean weight of 4.1 ± 0.5 kg (range, 3.56-5.46 kg). All procedures

were approved by the local review board (ComEth Anses/ENVA/UPEC n°16) and adhered to European legislation. Three groups of 6 were formed: non-operated (“non-op”), “early” with euthanasia 2 weeks after ACL sectioning, and “mid-early” with euthanasia at 6 weeks after ACL sectioning. The anesthetic and surgical techniques were as described previously [14]. Euthanasia was by barbiturate injection. Synovial fluid was sampled by arthrocentesis (20G needle) and conserved without additive at -80°C in individual hermetically sealed containers. Knee were explanted and conserved in gauze imbibed with 10x PBS (Phosphate Buffered Solution) in individual containers at -20°C until analysis. The medial part of the femoral condyle was selected for mechanical study, as known to be the most severely affected by ACL sectioning [15,16].

2.2 Mechanical study

Indentation tests, following Mäkelä et al. [16] (Figure 1), used an Agilent G200 nanoindenter, on 18 medial femoral condyles at 3 points in the weight-bearing region. Force was normalized to maximum force (3-35 mN) at the end of the penetration phase.

2.3 Poroelasticity

The indentation-relaxation curve shows how the interstitial fluid flows through the pores, assessing permeability (K). It also determines the shear modulus (G) corresponding to resistance to friction in the joint. Poisson’s coefficient (ν) corresponds to cartilage contraction perpendicularly to the force [17].

For the study, material behavior was assumed to be isotropic. Poroelasticity was assessed on bioMP² software (HealthDataSciences) based on an optimization algorithm in Python. For each sample, the 3 measurement points were analyzed independently.

2.4 Macroscopic analysis

Cartilage degradation and osteophytes were analyzed in double blind using the macroscopic grading system developed by Laverty et al. [18] (Table 1). After thawing at room temperature, synovial fluid volume was measured by pipette.

2.5 Statistics

Statistical analyses used R software (R Foundation for Statistical Computing, Vienna, Austria). Non-parametric tests were used to assess differences between independent groups. Intergroup comparisons were made on Kruskal-Wallis then post-hoc Mann-Whitney test with Benjamini-Hochberg adjustment for multiple comparison. Adjusted p-values less than 0.05 were considered significant.

3 Results

3.1 Poroelasticity

Table 2 and figure 2 show permeability, shear modulus and Poisson's coefficient. Permeability increased significantly ($p=0.007$) by a factor of 100 between the "non-op" and "early" groups then decreased significantly ($p=0.041$) by a factor of 10 in the "mid-early" group, while remaining significantly higher than in the "non-op" group ($p=0.039$). Shear modulus decreased significantly between "non-op" and "early" ($p=0.013$) and between "non-op" and "mid-early" ($p=0.023$). Poisson's coefficient decreased significantly only between the "early" group on the one hand and the "non-op" ($p=0.007$) and "mid-early" ($p=0.013$) groups on the other (Figure 2, Table 2).

3.2 Macroscopic scores

Table 3 and figures 3 show macroscopic scores per group. The "non-op" and "early" groups did not significantly differ. There were no osteophytes in the "non-op" group, and only 1 rabbit in the "early" group showed a small osteophyte on the femur and one on the trochlea. All rabbits in the "mid-early" group showed significant erosion of the intermediate cartilage layers ($p=0.011$) with osteophytes, sometimes severe, on the condyles and trochlea, with significantly higher macroscopic scores than in the "non-op" ($p=0.008$) or "early" group ($p=0.011$). Synovial fluid volume was too small for collection in the "non-op" group, but was considerable at 2 weeks ("early") and then decreased significantly ($p=0.030$) by a mean factor of more than 2 at 6 weeks ("mid-early").

4 Discussion

The present comparative animal study quantified a strong deterioration in cartilage poroelasticity in the days following modeling of ACL tear. Permeability in the non-operated group was comparable to the findings of Roemhildt et al. [19], but the association of strongly increased permeability and a considerable decrease in the mechanical properties of the femoral condyle in a very short period had never previously been reported. The study also showed easier flow of interstitial fluid from cartilage under loading, directly related to changes in extracellular matrix conformation.

These very considerable changes were accompanied by no obvious macroscopic degradation. Nakamura et al. [20] found the same trend in cartilage viscoelasticity, with significant change 2 weeks after ACL sectioning, well before any noticeable histologic alteration. When the cartilage is subjected to abnormal mechanical stress, the collagen matrix is altered, allowing the proteoglycan gel to absorb more water and thus increase in volume [21]. Cartilage swelling preceded degradation after ACL sectioning in dogs [22]. Increased cartilage thickness on MRI was reported to be an early sign of osteoarthritis in humans after ACL tear [23]. The present results showed loss of poroelasticity associated with visible cartilage degradation, in agreement with other reports [12,16].

Recent progress in biphoton microscopy [24] enables identification of cartilage microcracks, which are collagen microfissures. Unlike bone, cartilage has poor repair capacity [11], and microcracks may be the start of larger long-term fissures. In 2019, Han et al. [25] showed that cartilage relaxation was a major contributor to the formation of microcracks. In 2017, a team from the University of Connecticut [26] reported that low-energy impact could initiate microcracking, leading to a biological cascade and post-traumatic osteoarthritis. With the very poor mechanical properties found at early post-trauma time-points in the present study, the impact energy needed to cause microcracking would be much lower than in healthy cartilage. Data are also lacking in the literature for the effect of joint immobilization on the mechanical properties of cartilage under post-traumatic inflammation; if walking turns out to involve sufficient energy to initiate microcracks, non-weight-bearing might be advisable during the period in which the cartilage is especially at risk.

The present study had several limitations. It was an animal study, and might be completed by histologic analysis of cartilage to assess proteoglycan content and the possible link with macroscopic degradation and poroelasticity. The increase in synovial fluid volume seen at 2 weeks is related to osteoarthritis progression [27] and to postoperative inflammation due to ACL sectioning.

5 Conclusion

The very rapid degradation in poroelasticity observed in the present animal model of ACL tear needs combining with a biological analysis of the synovial fluid and analysis of microcrack nucleation to determine whether a change in management of patients treated for ACL tear, such as initial non-weight-bearing, might be beneficial for the joint cartilage.

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Disclosure of interest

Thierry Bégué, in relation to the present study, is an editor for OTSR, and elsewhere receives fees from Orthofix and Stryker. Raphael Vialle receives fees from Stryker, unrelated to the present study. The other authors have no conflicts of interest to disclose, in relation to the present study or elsewhere.

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Author contributions

JCA, Morad B, TH, CN, Manon B: study conception and design; Morad B, TH, Manon B: preparation and execution of surgeries; CN, TH: data acquisition; CB: statistical analysis. All authors: article writing, critical revision and final approval.

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Figure legends

Figure 1: 1a: Indentation-relaxation tests on femoral samples, on 2 views. Black line: sample cut plane; blue arrow: indentation direction; hatched area: indentation zone. 1b: Typical relaxation curves in “non-op”, “early” and “mid-early” groups.

Figure 2: Medial femoral condyle poroelasticity. 2a: Permeability. 2b: Shear modulus. 2c: Poisson’s coefficient. *: significant intergroup difference.

Figure 3: 3a and 3b: Macroscopic scores following Laverty et al. [18]: femoral cartilage degradation and osteophyte. 3c: Volume of collected synovial fluid. *: significant intergroup difference.

Table 1: Osteoarthritis progression scores (Lavery et al. [18]).

Joint cartilage (tibial and femoral condyles)
0: Surface smooth with normal color
1: Surface rough with slight fibrillation or slightly yellowish color
2: Cartilage erosion extending to superficial or intermediate layers
3: Cartilage erosion extending to deep layers
4: Complete cartilage erosion exposing subchondral bone
Osteophyte formation (tibial plateau, femoral condyles and trochlea)
0: None
1: Mild
2: Moderate
3: Severe

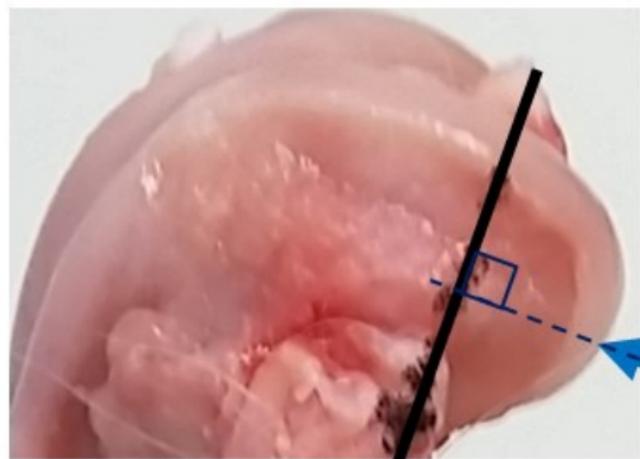
Table 2. Medial femoral condyle poroelasticity per group: mean \pm SD and p-values

Group	non-op	early	mid-early	p-values		
				non-op/early	non-op/mid-early	early/mid-early
Permeability K ($\times 10^{-15} \text{ m}^4/\text{Ns}$)	0.08 \pm 0.05	1.03 \pm 0.60	0.33 \pm 0.33	0.007	0.039	0.041
Shear modulus G (MPa)	0.53 \pm 0.11	0.23 \pm 0.10	0.30 \pm 0.10	0.013	0.023	NS
Poisson's coefficient ν	0.48 \pm 0.01	0.42 \pm 0.03	0.47 \pm 0.02	0.007	NS	0.013

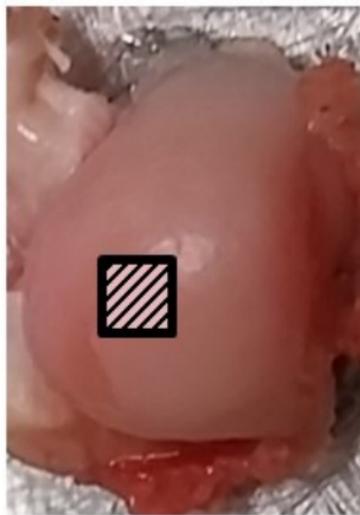
Table 3. Macroscopic scores: mean±SD and p-values

Group	non-op	early	mid-early	p-values		
				non-op/early	non-op/mid-early	early/mid-early
Cartilage degradation	0.17±0.37	0.17±0.37	1.17±0.37	NS	0.011	0.011
Osteophytes	0.00±0.00	0.33±0.75	3.00±1.15	NS	0.008	0.011
Synovial fluid volume (µl)	NS	230±70	112±75			0.30

(a)



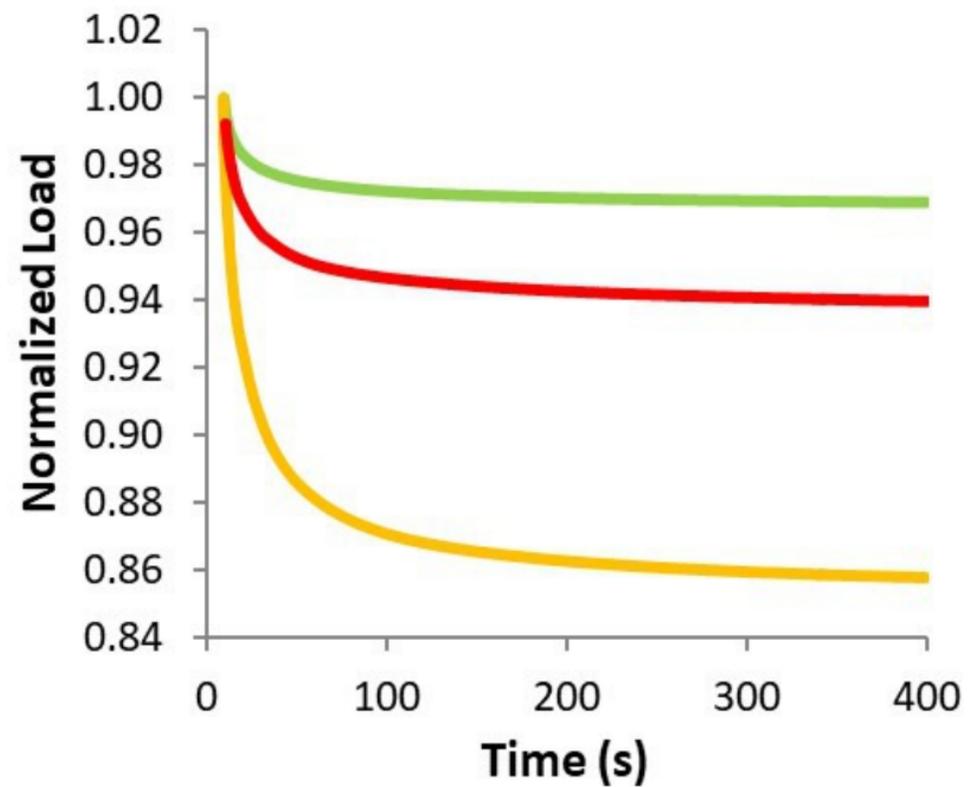
Indentation
direction

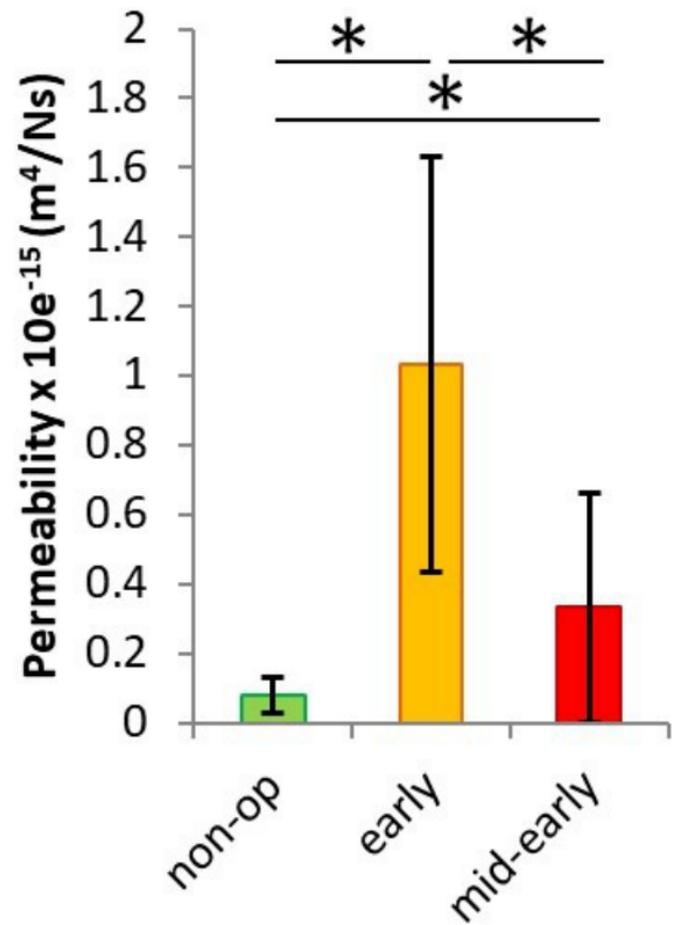


(b)

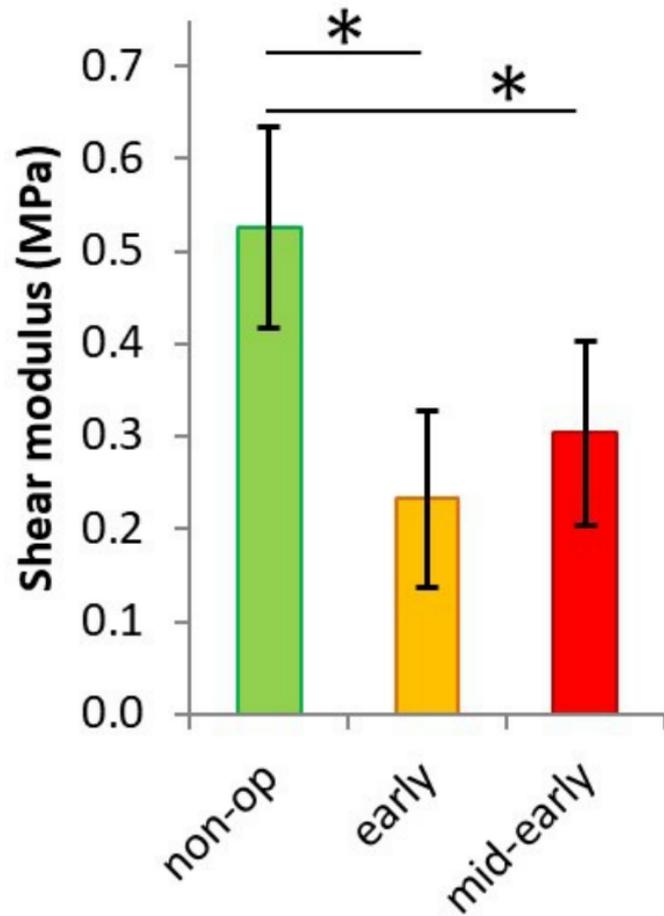
Indentation relaxation test

— non-op — early — mid-early

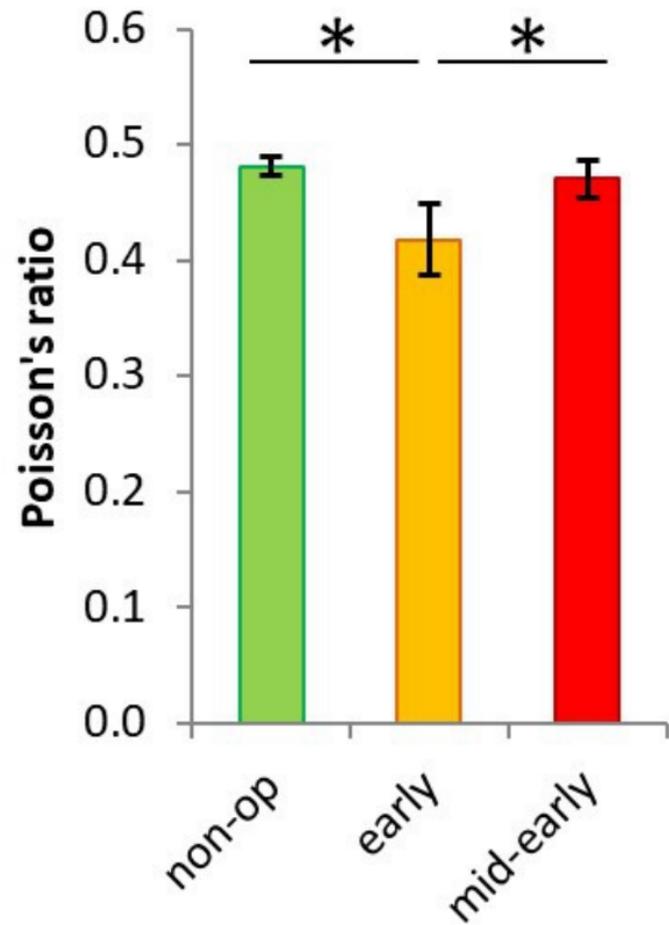




(a)

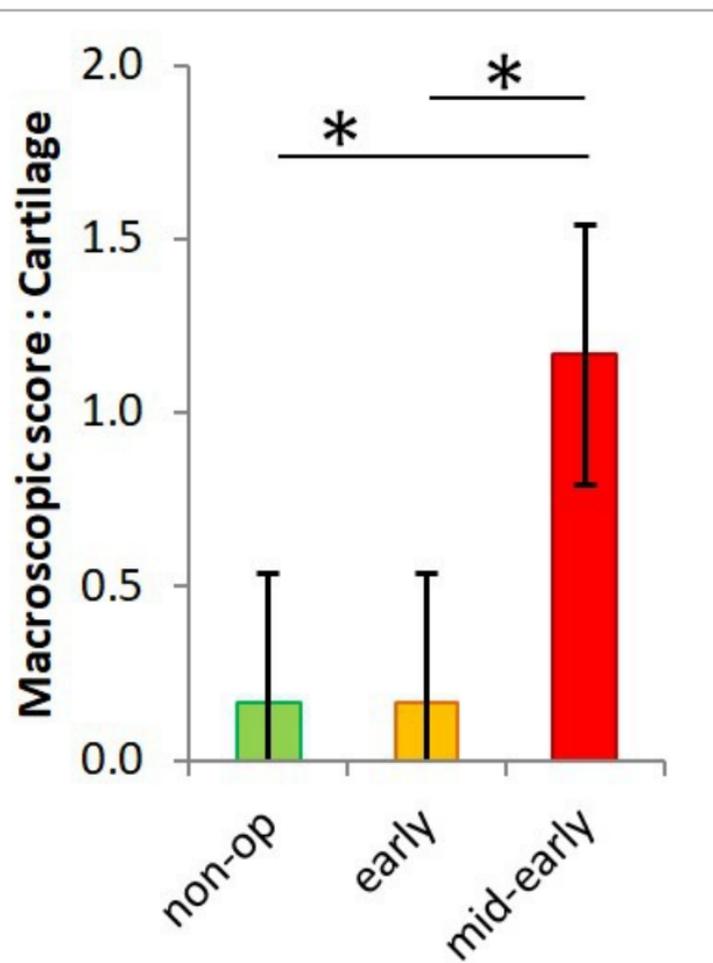


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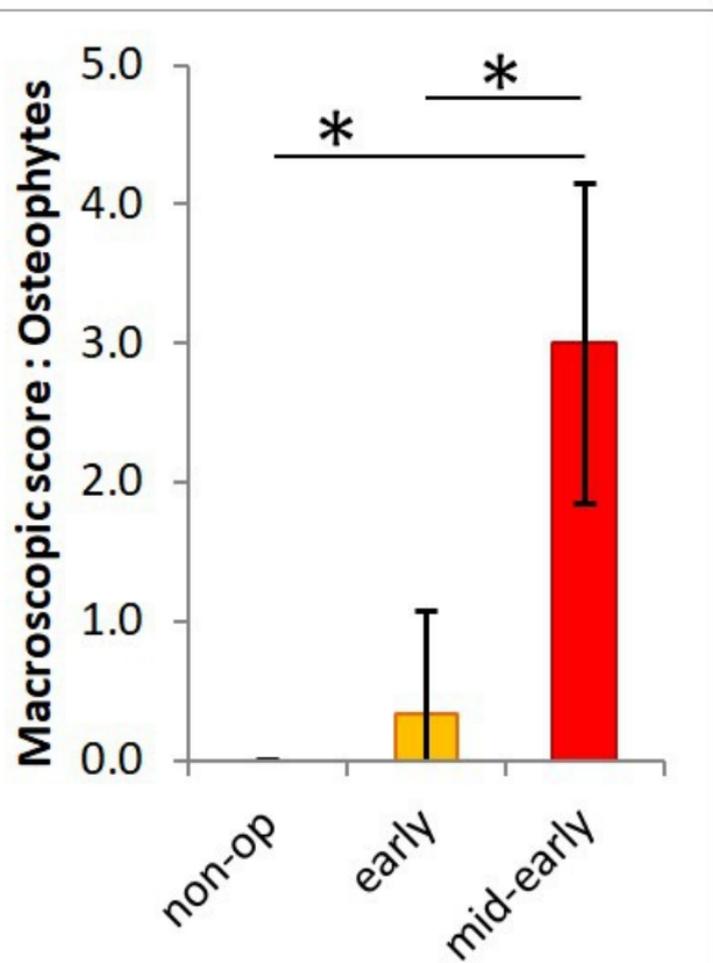


(c)

(a)



(b)



(c)

