第61卷 第2期
2023年4月

古 脊 椎 动 物 学 报
VERTEBRATA PALASIATICA

pp. 108–122
figs. 1–4

DOI: 10.19615/j.cnki.2096-9899.230309

Do chondrocytes within calcified cartilage have a higher preservation potential than osteocytes? A preliminary taphonomy experiment

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Abstract Chondrocytes with remnants of nuclei and biomolecules were recently reported in two Cretaceous dinosaurs from North America and China. For multiple reasons, it was hypothesized that calcified cartilage (CC) had a better potential than bone to preserve ancient cells. Here we provide the first experimental test to this hypothesis by focusing on the most important variable responsible for cellular preservation: the postmortem blockage of autolysis. We compare the timing of autolysis between chondrocytes and osteocytes in an avian model (*Anas platyrhynchos domesticus*) buried for up to 60 days under natural conditions that did not inhibit autolytic enzymes. Within 15 days post-burial, almost all osteocytes were already cytolyzed but chondrocytes in CC were virtually unaffected. All osteocytes were cytolyzed after 30 days, but some chondrocytes were still present 60 days post-burial. Therefore, even in harsh conditions some CC chondrocytes still survive for months postmortem on a time scale compatible with permineralization. This is consistent with other data from the forensic literature showing the extreme resistance of hyaline cartilage (HC) chondrocytes after death and does support the hypothesis that CC has a better potential than bone for cellular preservation, especially in fossils that were not permineralized rapidly. However, because the samples used were previously frozen, it is possible that the pattern of autolysis observed here is also a product of cell death due to ice crystal formation and not strictly autolysis, meaning a follow-up experiment on fresh (non-frozen samples) is necessary to be extremely accurate in our conclusions. Nevertheless, this study does show that CC chondrocytes are very resistant to freezing, suggesting that chondrocytes are likely better preserved than osteocytes in permafrost fossils and mummies that underwent a freezing-thawing cycle. It also suggests that cartilage (both hyaline and calcified) may be a better substrate for ancient DNA than bone. Moreover, even though we warrant follow-up taphonomy experiments with non-frozen samples paired with DNA sequencing, we already urge ancient DNA experts to test CC as a new substrate for ancient DNA analyses in fossils preserved in hot and temperate environments as well.

国家自然科学基金(批准号：42288201, 42172029)资助。
收稿日期：2022-12-02

Key words experimental taphonomy, chondrocytes, osteocytes, postmortem autolysis, freeze related cell-death, cellular and biomolecule fossilization, aDNA substrate

Citation Bailleul A M, Wu Q, Li D S et al., 2023. Do chondrocytes within calcified cartilage have a higher preservation potential than osteocytes? A preliminary taphonomy experiment. Vertebrata PalAsiatica, 61(2): 108–122

1 Introduction

Seventy-five million-year-old chondrocytes preserved with their fossilized nuclei and chromosomes where recently reported in the calcified cartilage (CC) of the North American dinosaur *Hypacrosaurus* (Bailleul et al., 2020). Additionally, two fluorescent DNA stains were applied to a few *Hypacrosaurus* chondrocytes that were isolated from their extracellular matrix (ECM) and a few cells showed fluorescent intracellular DNA staining (Bailleul et al., 2020). More recently, a cell nucleus with chromatin-like threads that stained just like an extant avian nucleus with hematoxylin and eosin was reported in the CC of a 125 million-year-old oviraptorosaurid dinosaur (*Caudipteryx*) from Northeast China (Zheng et al., 2021).

The results of these two studies suggested that some cell nuclei from Mesozoic cartilage cells still retain remnants of original nuclear biomolecules (Bailleul et al., 2020; Zheng et al., 2021). Even though this type of studies and results are highly controversial (Schweitzer et al., 2013; Bailleul et al., 2020; Zheng et al., 2021) because DNA sequencing has never yielded an authenticated sequence from any fossil this old (van der Valk et al., 2021) the data still suggest remnants of original DNA are preserved, even if the DNA might be chemically modified (Bailleul and Li, 2021).

Data from *Hypacrosaurus* led to the hypothesis that CC may be a better candidate than bone for cellular and biomolecule preservation. This hypothesis was based on noticeable tissue characteristics present only in cartilage and which seemed to be favorable to molecular stabilization. For example, unlike bone, the ECM of cartilage (both uncalcified and calcified) has no vascularization, which gives less surface area available for ground water and microbes to deteriorate the tissue. CC also has a higher mineral:organic ratio than that of bone (Goret-Nicaise and Dhem, 1985), presumably giving further protection for the chondrocytes from external attacks. Lastly, CC is a hypoxic environment (with sometimes only 1% of oxygen (Pfander and Gelse, 2007)), and the authors (Bailleul et al., 2020) proposed this hypoxia may protect chondrocytes from oxidative damage.

It was later noted that an additional key characteristic of chondrocytes further supported this hypothesis: the fact that chondrocytes naturally have an extensive postmortem delay of autolysis (Bailleul, 2021), at least for chondrocytes in uncalcified hyaline cartilage (HC) (Csönge et al., 2002; Lasczkowski et al., 2002; Williams et al., 2003; Drobnic et al., 2005; Pallante et al., 2009; Rogers et al., 2011; Alibegović, 2014; Paulis et al., 2016). Autolysis is the autodigestion of cells by their own lytic enzymes after somatic death, a process that culminates

into cytolysis (the complete breakdown of all cell components). It usually starts extremely rapidly within a few hours after death in most cell types and is dependent on the metabolic rate (high or low) of the cell (Clark et al., 1997; Powell, 2015). Cells with a high metabolic rate get autolyzed the fastest (Clark et al., 1997; Powell, 2015). During what is referred to as 'exceptional' cellular fossilization, autolysis is blocked by external factors (e.g., a rapid burial in an anoxic environment combined with reducing conditions that denature hydrolytic enzymes; or cold temperatures in permafrosts) (Raff et al., 2006, 2008). Without the blocking of autolysis, or at least a delay of autolysis long enough to allow permineralization and/or mineral replication (Briggs et al., 1993), cells and their biomolecules are not expected to enter the fossil record (Raff et al., 2006, 2008; Bailleul and Li, 2021).

Research on articular HC allograft taken from human cadavers and transplanted into live patients with osteoarthritic joints have shown that many chondrocytes retain their viability for long periods after somatic death (Csönge et al., 2002; Lasczkowski et al., 2002; Williams et al., 2003; Drobnic et al., 2005; Pallante et al., 2009). Chondrocytes function with an anaerobic metabolism, are adapted to live in a hypoxic environment and can survive in modest conditions low in both nutrients and oxygen (even in full anoxia (Pfander and Gelse, 2007)). After somatic death, the nutrients and oxygen stored in the avascular ECM of cartilage still diffuse to the HC chondrocytes, allowing their survival several days -to weeks or even months- after the individual's death (Csönge et al., 2002; Lasczkowski et al., 2002; Williams et al., 2003; Drobnic et al., 2005; Pallante et al., 2009; Alibegović, 2014).

Such an exceptional timing of postmortem survival is apparently not seen to this extreme in any other tissue of the human body (Pfander and Gelse, 2007). Unfortunately, even though abundant data on the postmortem timing of autolysis in mammalian HC are available (Csönge et al., 2002; Lasczkowski et al., 2002; Williams et al., 2003; Drobnic et al., 2005; Pallante et al., 2009; Rogers et al., 2011; Alibegović, 2014; Paulis et al., 2016), virtually nothing is known about CC for any vertebrate taxon. Although this was never clearly stated nor demonstrated, we can assume that osteocytes die and get autolyzed much more rapidly than both HC and CC chondrocytes after death, since the former function with an aerobic metabolism under basal conditions (Shapiro et al., 1982; Frikha-Benayed et al., 2016) - directly receiving nutrients and oxygen from blood vessels - and are not adapted to naturally thrive under hypoxia. This was already assumed and proposed in a few studies (Bailleul, 2021; Bailleul and Zhou, 2021; Zheng et al., 2021) but 1) it was never clearly tested in a comparative setting with unequivocal histological images; and 2) contradicting data exist concerning bone cells, with a forensic study stating that the cells of 'skeletal tissues' (presumably bone cells) are some of the last ones to autolyze (Clark et al., 1997).

Here, we perform a simple actualistic taphonomy experiment comparing the timing of autolysis between CC and bone in an avian model which can serve as a baseline for inferences concerning cellular and biomolecule fossilization in these two tissues and will help test the original hypothesis that CC is a better candidate than bone for ancient cells and biomolecule

preservation. The experiment was made in harsh conditions that are not comparable to 'exceptional' fossilization (i.e., buried during the summer, under oxic conditions, without antibiotics) to observe the 'natural' autolytic behavior of chondrocytes and bone cells after death. For an additional element of comparison, we also decided to analyze the timing of autolysis in HC for which considerable data has been generated recently from the fields of clinical medicine and forensics. We used the webbed feet of Domestic ducks (*Anas platyrhynchos domesticus*) that were buried for up to 60 days and we histologically analyzed and compared the cells of the three tissues at the joint between the distal end of the third tarsometatarsus (TMT III) and the third proximal phalanx.

2 Materials and methods

2.1 Materials

Three specimens of Domestic ducks of unknown sex and 50 days-old (~7 weeks) were obtained from a commercial bird farm located in Linyi (Shandong Province). Specimens were close to skeletal maturity (bone growth being complete at 8 weeks of age in this species (Murawska, 2012)). Specimens were sacrificed according to local permits of the farm, then immediately transferred into an −18ºC freezer (within 1 hour of death). After a few days in this freezer they were shipped frozen to Beijing (~16 hour drive) and placed into another freezer at the Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences (IVPP). The ducks were still frozen upon arrival. In total, the ducks stayed frozen at −18ºC for 26 days until the start of the experiment (at t = 0 days).

2.2 Experimental setting

After 26 days in the freezer, three ducks were taken out and their frozen feet and heads were sawed (although note that the heads in Fig. S1 were buried for the purpose of another study). One foot of the first duck (Duck 1) was used as the control foot and was processed directly for histology after having thawed (Supplementary methods). The sawed parts of the other two ducks (Duck 2 and Duck 3) were then transported and buried approximately 20 cm deep in the soil (a sandy loam of pH ~ 7.8) of a nearby compound at the Beijing Zoo (Fig. S1). Elements were placed approximately 10–15 cm apart in the soil (Fig. S1). The experiment took place between July 1st 2020 and September 29th 2020. The average precipitation for these 3 months was 128.2 mm and the average temperature was 23.2ºC in Beijing (source: http://www.nmc.cn/publish/forecast/ABJ/beijing.html).

2.3 Paraffin histology

For the control foot of Duck 1 and the excavated feet of Duck 2 and 3, tissue samples were cut off from the articular surface of the TMT III at the tarsometatarsophalangeal joint with a sharp razor blade. They were then put into 10% Neutral Buffered Formalin and processed to be transformed into paraffin slides and stained with H&E and a modified Masson's Trichrome (Supplementary methods).

2.4 Scanning electron microscopy

Some finished paraffin slides were deparaffinized and left to dry without a coverslip. These deparaffinized sections were then sprayed with gold, then observed and photographed rapidly under the SEM at the Chinese Academy of Geological Sciences with an FEI Quanta 450 (FEG) at 20 kV. Images are shown in the BSE mode (backscattered electrons).

3 Results

3.1 Gross morphology

One frozen-thawed duck foot was not buried and used as the control foot for this experiment (at t = 0 days) (Fig. 1A). Three other (previously frozen) feet were buried at the same time but excavated at three different times: one after 15 days, one after 30 days, and one after 60 days in the soil (Figs. 1B–D; S1). In the control foot, the skin was bright yellow and the articular HC on the TMT III was transparent and glossy (Fig. 1A). After 15 days in the soil, the skin around the analyzed joint lost its bright yellow color and the HC turned white and cloudy (Fig. 1B). After 30 days, the soft tissues and skin dried out and the HC was lost,

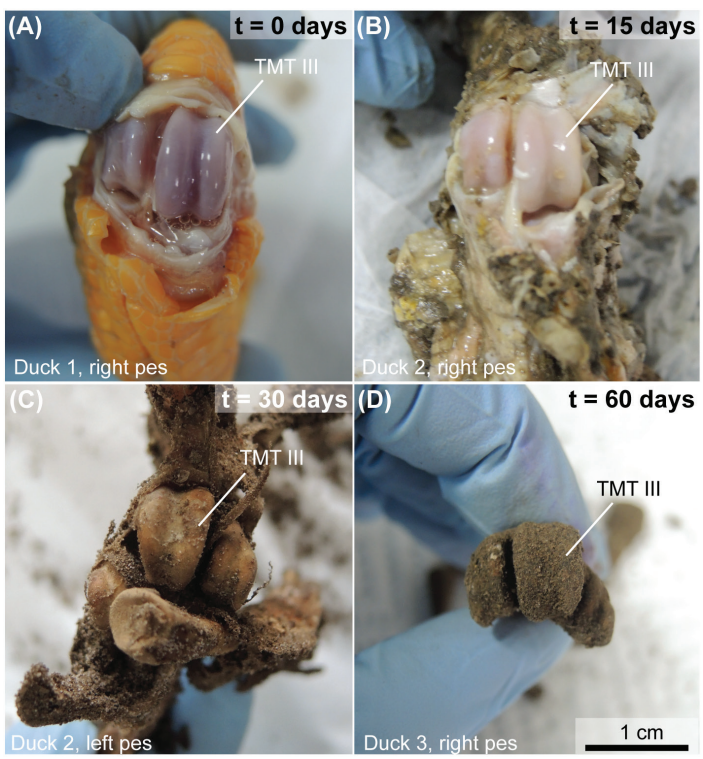

Fig. 1 Changes in the gross morphology of the tissues at the tarsometatarsophalangeal joint of the control and buried feet

A. tissues of the control foot (at t = 0 days); B. decayed tissues of a foot buried for 15 days; C. decayed tissues of a foot buried for 30 days, loss of calcified cartilage is apparent; D. decayed tissues of a foot buried for 60 days. Images are at the same scale. Abbreviation: TMT III. third tarsometatarsal

only leaving exposed CC and subchondral bone (Fig. 1C). After 60 days in the soil, all the surrounding soft tissues disappeared and the bones of the foot were all disarticulated (Fig. 1D).

3.2 Histology and SEM changes from t = 0 days to t = 15 days

At t = 0 days, the chondrocyte lacunae of the HC were filled with large chondrocytes that stained dark purple with hematoxylin and eosin (H&E) (Fig. 2C). At t = 15 days, the

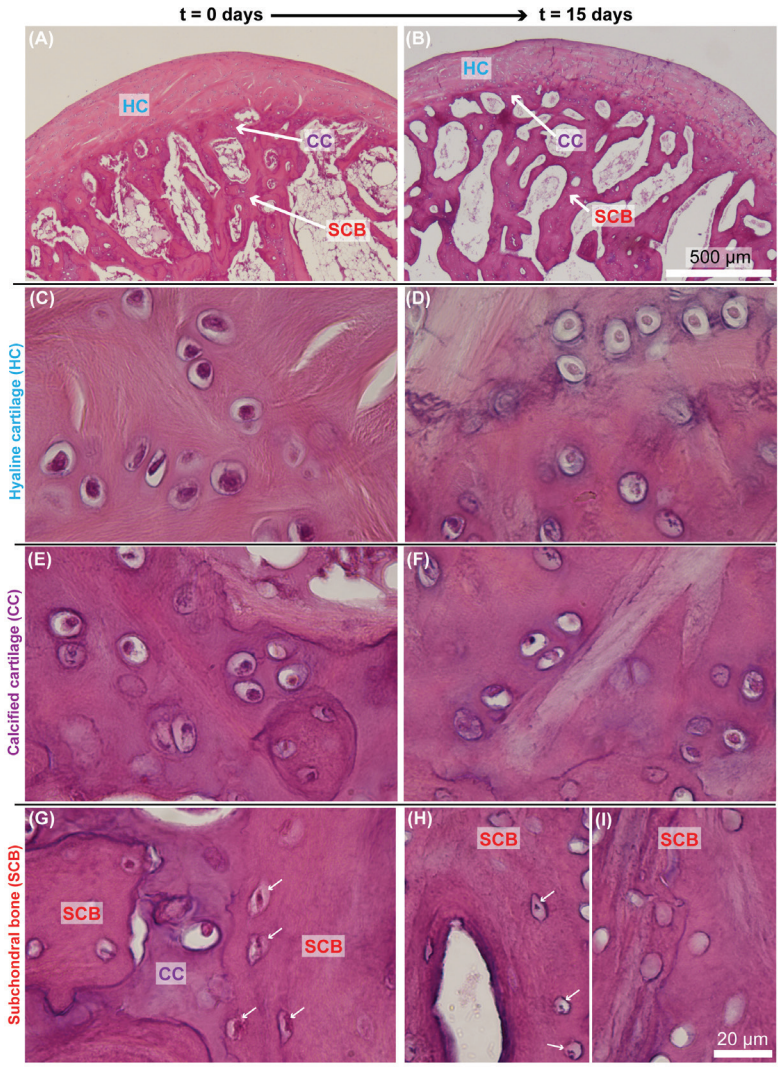

Fig. 2 H&E stained paraffin slides of the control foot (A, C, E, G) and the foot buried for 15 days (B, D, F, H, I) The cells in hyaline cartilage (HC) are round and stain dark purple (C); after 15 days in the soil, the chondrocytes shrank and lost their nuclear basophilia (D); control chondrocytes embedded in CC (E); after 15 days in the soil, the CC chondrocytes retained their nuclear basophilia and were virtually unchanged (F); control osteocytes within osteocyte lacunae (white arrows) in the subchondral bone (SCB) (G); remnants of autolyzed osteocytes (white arrows, with full cytolysis not attained yet) (H); empty osteocyte lacunae (meaning cytolysis was complete within 15 days postmortem) (I) A, B are at the same scale; C–I are at the same scale

chondrocytes had considerably shrunk and had become light pink (meaning that they did not take up as much stain as those of the control foot; Fig. 2D). This loss of nuclear basophilia is known to occur in the cells of decomposing remains stained with H&E (Genest et al., 1992; Rogers et al., 2011; Dettmeyer, 2018; Alabbasi et al., 2022) and is due to autolysis (Dettmeyer, 2018; Alabbasi et al., 2022). It is likely that chromatin dissolution had started and that the HC chondrocytes were no longer viable at t = 15 days.

SEM observations show that these cells at t = 0 days were round and mostly smooth (Fig. 3A), but the shrunken, hypothetically non-viable cells of t = 15 days adopted a stellate shape with many 'protrusions' (Fig. 3B). The latter may be extra-cellular secretions or breakdown products from autolysis itself.

In the CC, no clear differences in staining intensity, cell shape nor cell size could be seen

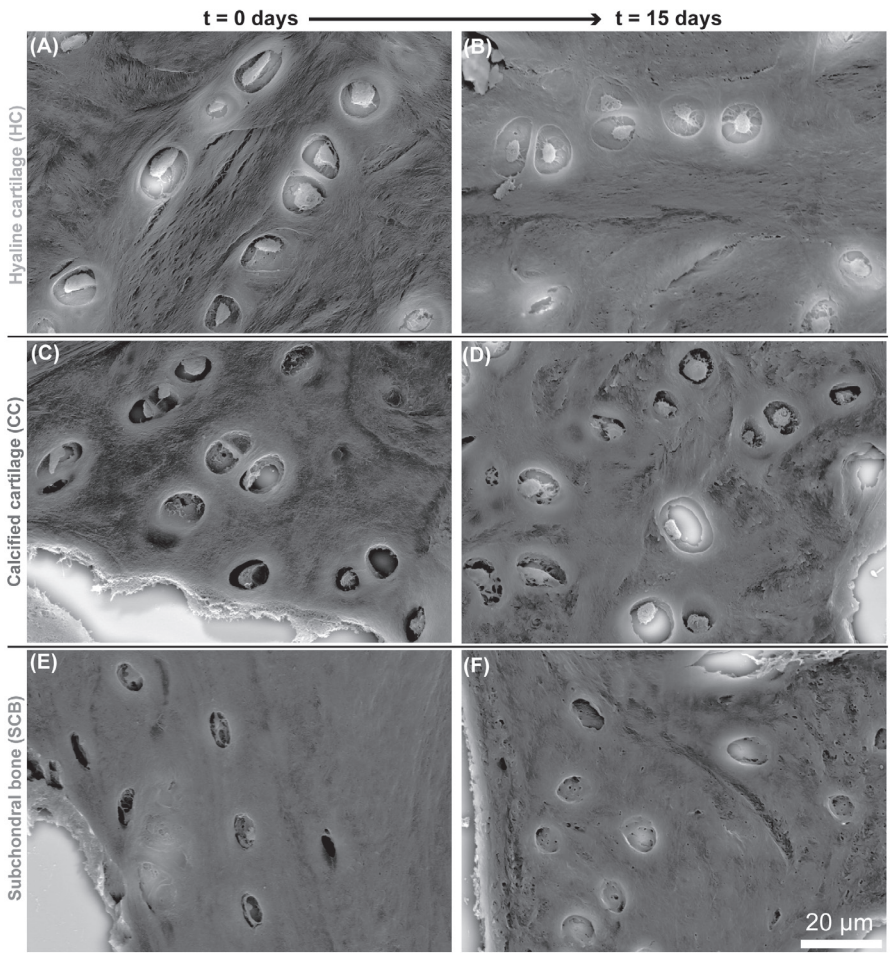

Fig. 3 SEM images of the control tissues (A, C, E) and the foot buried for 15 days (B, D, F)
A. the cells in HC are large and round; B. after 15 days in the soil, they are shrunken and show filamentous protrusions; C. morphology of the control chondrocytes embedded in CC; D. the morphology of the CC chondrocytes remained unchanged; E. osteocytes inside bone lacunae; F. osteocyte lacunae completely empty
All images are at the same scale

between t = 0 days and t = 15 days (Fig. 2E, F): in both cases, these cells were round and dark purple. No obvious differences in surface morphology could be seen under the SEM either (Fig. 3C, D), suggesting the CC cells were not affected by somatic death and were most likely still viable at t = 15 days, unlike the chondrocytes of the HC (Figs. 2C, D, 3A, B).

The subchondral bone has round osteocyte lacunae in many places resembling chondrocyte lacunae. This is a characteristic of chondroid bone, a bony tissue quite common in rapidly growing juvenile ducks (Prondvai et al., 2020). At t = 0 days, bone cells from the subchondral bone showed a healthy and elongated morphology (Fig. 2G).

At t = 15 days, many osteocyte lacunae of the subchondral bone were completely empty, meaning that many cells had already been fully cytolyzed within 15 days post-burial. (Figs. 2I, 3F). The rest of the osteocyte lacunae were filled with small round bodies representing autolyzed remnants that had not yet been fully cytolyzed (Fig. 2H).

3.3 Histological changes from t = 30 days to t = 60 days

No clear differences were seen between the decayed tissues of t = 30 days and t = 60 days. All the HC had disappeared by putrefaction and only CC and subchondral bone were present (Fig. 4A, B). Most CC lacunae were either empty or filled with contaminating agents

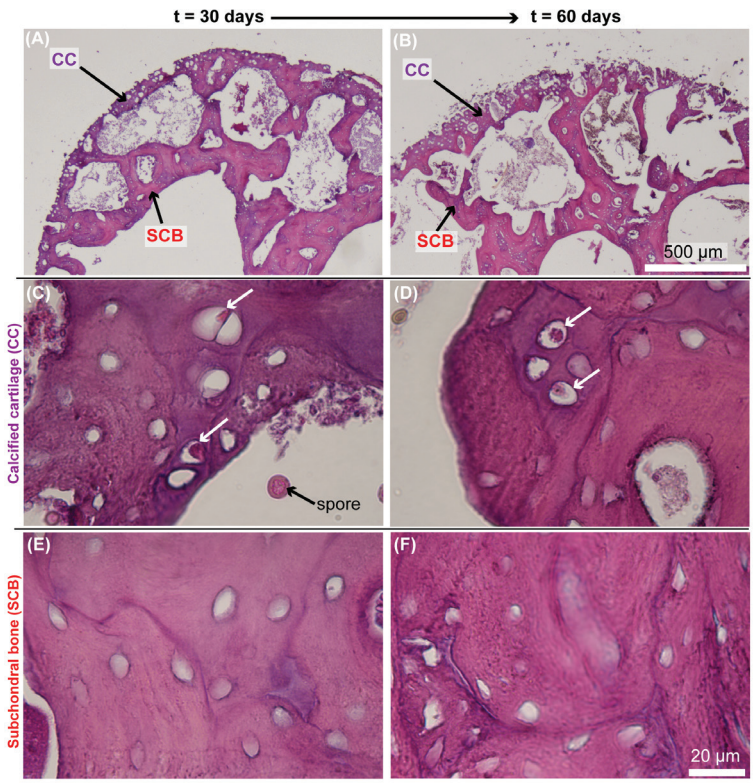

Fig. 4 H&E stained paraffin slides of the foot buried for 30 days (A, C, E) and that buried for 60 days (B, D, F)
A, B. CC and SCB; C, D. CC, some chondrocytes are still found within their lacunae (white arrows) and retained nuclear basophilia, and the same result can be seen in D (white arrows); E, F. SCB, shows osteocyte lacunae that are completely empty in E, and the same observation can be seen in F
A, B are at the same scale; C–F are at the same scale

like spores (as seen in both paraffin slides and SEM observations, Fig. S2), but a few lacunae still showed round chondrocytes that stained light to dark purple (Figs. 4C, D; S3). The dark purple stain means nuclear basophilia was still retained after 30, and even 60 days post-burial, suggesting that a few last 'surviving' CC cells may still have been viable (or at least they may have been 'damaged' cells *sensu* Lasczkowski et al. (2002) - but not fully dead).

In the subchondral bone, no remnant of osteocyte could be seen in any of the osteocyte lacunae at either t = 30 days or t = 60 days (Fig. 4E, F).

4 Discussion

4.1 First observed pattern: bone cells autolyzed faster than cartilage cells

At t=15 days, an obvious pattern of autolysis between the three cell types analyzed could be discerned: almost all the bone cells were fully cytolyzed, the HC cells were still present but showed a loss of nuclear basophilia (a sign of karyolysis (George et al., 2016)), and the CC cells were virtually the same in morphology, size, and staining intensity as those of the control foot (Fig. 2). This means a clear gradient exists in the timing of autolysis of osteocytes and different types of chondrocytes in our samples, with bone cells being the fastest to start and to reach full autolysis, followed by HC cells, and finally CC cells. We can confidently estimate that - within 1 week in the soil - at least 50% of the osteocytes were already completely cytolyzed in our samples. An inhibition of autolysis for months post-burial (as seen in some CC chondrocytes here) is largely compatible with the timing of permineralization, which can happen within just a few weeks in experimental studies (e.g., ~2–3 weeks (Martin et al., 2003, 2005; Chen et al., 2009)).

The differential timing of autolysis seen in these three tissues can be explained by differences in the biology and metabolism of their cells. As we predicted, autolysis was more rapid in osteocytes than in HC and CC chondrocytes, and this is likely due to the fact that bone cells are not naturally adapted to hypoxia. However, we did not predict that CC cells would be even more resistant to postmortem changes than HC cells. Even though quantitative and comparative data are lacking, HC cells are generally reported as being the cell type with the most exceptional timing of postmortem survival in the body (Pfander and Gelse, 2007) and chondrocytes in CC are usually thought to die quite rapidly once they are embedded in a calcified matrix. This latter assumption is simply erroneous, nutrients and solutes can still diffuse through the calcified cartilaginous matrix (Arkill and Winlove, 2008) and our study shows that CC chondrocytes have a life span of at least few months. Our current results re-attribute the postmortem survival 'record' of HC chondrocytes to chondrocytes embedded in CC.

It has already been demonstrated that the layers of HC that are closer to the articular surface are more affected by postmortem environmental factors than the deeper layers of articular cartilage (i.e., closest to the subchondral bone) (Drobnic et al., 2005). Moreover, a

recent study showed that chondrocytes of the deeper layers are more adapted and resistant to hypoxia (with a hypoxia-adapted phenotype) than the chondrocytes closer to the surface (Brucker et al., 2005; Pfander and Gelse, 2007). Based on this information, we hypothesize that CC chondrocytes have a unique hypoxia-adapted phenotype allowing them to have a postmortem survival period that surpasses that of all HC cells (and of course, bone cells).

It can be extrapolated that cells (including their biomolecules) having an extended postmortem survival period (i.e., cells in which autolysis is blocked, or not activated for long periods) technically have more chances to enter the fossil record than cells that get autolyzed more rapidly after death. The pattern of autolysis observed here is therefore in favor of our original hypothesis that CC is a better candidate than bone for ancient cells and biomolecule preservation.

However, this is a preliminary conclusion because our tissues were frozen before the experiment (at −18ºC for 26 days), meaning that the results reported here may also show an unrepresentative pattern of decay due to freeze-related cell death. Indeed, freezing causes mechanical cell damage due to the formation of intra- and extracellular ice crystals (Jang et al., 2017) and even though highly unlikely, we cannot technically rule out the possibility that osteocytes may be more sensitive to cold than chondrocytes. Cell damage was indeed seen in some cells of the control tissues (Fig. S4), meaning that more appropriate material for this type of histotaphonomy experiment is fresh, non-frozen tissues in which the original cell viability is not affected by freeze-thaw cycles. The samples should also be sent for DNA sequencing analyses to test for the correlation between cell integrity/presence and DNA/biomolecule preservation because it has been hypothesized that molecules of DNA can ‘leak’ from cells and be adsorbed and retained within crystals of hydroxyapatite after death (Götherström et al., 2002).

4.2 Second observed pattern: CC chondrocytes are quite resistant to freezing temperatures

Even though the material used in this experiment does not allow for a full support of the primary hypothesis tested here, our results nevertheless do show that CC chondrocytes are quite resistant to very low temperatures. This taphonomical experiment may therefore serve as a preliminary model for permafrost mummies that go through freezing-thawing cycles (with temperatures usually varying between 0ºC and −15ºC; e.g., Sazonova et al (2004)); our results suggest that the chondrocytes of both HC and CC of permafrost-preserved fossils and mummies may be better preserved than their osteocytes. Since DNA is found within cells, it is likely that cartilage is a better substrate for ancient DNA (aDNA) than bone in permafrosts mummies.

5 Perspectives

Taphonomy experiments which aim to better understand and interpret fossilization processes rarely involve histology even though this method provides a deeper understanding

of tissues and their cells than gross examinations. The histotaphonomical results of this experiment bridge multiple fields of study including taphonomy, paleontology and potentially the field of aDNA.

In the field of aDNA where the current limit of sequence authenticity is only around 1.2~1.65 million years old (van der Valk et al., 2021), great efforts are constantly being made to identify the best high-purity 'substrates' for aDNA recovery and the skeletal elements that are the richest in DNA in a given taxon (e.g., Götherström et al., 2002; Hansen et al., 2017; Alberti et al., 2018). The best 'substrates' are tissues and parts of the body that have the highest percentage of endogenous aDNA and the lowest percentage of contaminating DNA (Hansen et al., 2017; Sirak et al., 2020). In recent years, studies have shown that the most suitable substrates for DNA analysis in ancient humans preserved in hot and temperate environments are the cementum of teeth roots (Hansen et al., 2017), the petrous part of the temporal bone (Pinhasi et al., 2015), and the auditory ossicles (Sirak et al., 2020). How and where DNA is found within these high-purity tissues is not clearly understood, but hypothetically the petrous bone is richer in DNA than other part of the body because 1) it is the densest bone of the human skeleton and is somewhat protected from taphonomical processes (Gamba et al., 2014; Pinhasi et al., 2015), and 2) it has a high number of micropetrotic mineralized osteocytes which supposedly help sequester DNA molecules (like 'microniches' of DNA (Bell et al., 2008; Pinhasi et al., 2019)). This type of cell mineralization is also possible within CC (see references in Kierdorf et al. (2022)); recently some calcified 'micropetrotic' chondrocytes were reported in the CC of the fossil bird *Yanornis* (Bailleul and Zhou, 2021).

To our knowledge, CC is not a tissue that has clearly been tested as a potential high-purity substrate for aDNA analysis in neither permafrost fossils nor those preserved in hot and temperate environments even though it has all the tissue architecture and proper cell metabolism to be such a substrate. Based on the knowledge of the cold-resistance of CC cells reported in this study combined with the fact that CC is avascular (so it can be considered 'dense') with a high cellular density, there is no reason not to sample this tissue in permafrosts fossils. Additionally, pending other taphonomical experiments using fresh (non-frozen) material, our preliminary data still suggest that CC has the potential to be a new high-purity aDNA substrate (possibly providing better results than dense micropetrotic bone or cementum) for fossils preserved in hot and temperate environments as well.

We propose that fragments of CC covering the joints of adult humans, or the basicranial synchondroses of infants should be sampled when possible and that their DNA yield should be compared to that of other tissues. This would be especially important in fossils where bones fragments or cementum did not yield good results (only very incomplete DNA sequences). We also anticipate that CC could have a significantly higher DNA purity than bone in large species (such as large Pleistocene mammals) that naturally have a very thick layer of CC at their joints. Additionally, fossil chondrichthyans (which are entirely made of cartilage) may also be great study material to further investigate these interesting questions about DNA preservation.

Acknowledgements We thank Wang Yan for providing the duck specimens and helping with their shipment to Beijing. For discussions we thank Pan Yanhong, Dana Rashid, Brian K. Hall, Magdalena Koziol. We also thank Pan Yanhong and one anonymous reviewer for reviewing this manuscript. This work was funded by grants NSFC 42288201, 42172029.

Supplementary file can be found on the website of Vertebrata PalAsiatica (http://www.vertpala.ac.cn/EN/2096-9899/home.shtml).

钙化软骨中的软骨细胞比骨细胞具有更高的保存潜力? 埋藏学实验初探

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摘要：近期，在发现于美国和中国的两例早白垩世恐龙中报道了保存有细胞核物质和生物分子的软骨细胞的存在。基于多种原因，研究人员认为，钙化软骨比骨骼更有可能保存远古细胞。通过针对影响细胞保存的最主要因素：机体死亡后细胞自溶过程的停止，对这一假说进行了首次实验验证。以家鸭 (*Anas platyrhynchos domesticus*)为模型，比较了在不抑制自溶酶的情况下，自然埋藏60天内，软骨细胞和骨细胞的自溶过程。埋藏后的15天内，几乎所有骨细胞均发生了自溶，而钙化软骨细胞则基本未受影响。埋藏30天后，所有骨细胞均已自溶，但一些软骨细胞在埋藏60天后仍然存在。因此，即使在恶劣的条件下，钙化软骨细胞仍然能够在动物机体死亡后存活数月之久，而这一时间足以实现矿化过程。这一结果与一些法医文献中的数据相吻合，表明透明软骨细胞在机体死亡后能够长时间抵抗分解，且支持了钙化软骨比骨骼更有保存细胞结构的潜力的假说，尤其是在未能快速矿化的化石中。然而，由于所使用的标本预先经过冷冻，所观察到的自溶模式也有可能是细胞由于冰晶形成而死亡的结果，而非严格的自溶过程，因此有必要进一步对新鲜标本开展实验以提高结论的准确度。无论如何，研究明确显示透明软骨和钙化软骨的软骨细胞受到冷冻的影响比骨细胞更小。这暗示软骨细胞比骨细胞更可能在发现于永久冻土的化石或木乃伊中保存下来，而软骨(包括透明软骨和钙化软骨)可能是比骨骼更为理想的古DNA研究对象。尽管有必要对未经冷冻的新鲜样品进行后续实验并辅以DNA测序，建议古DNA专家将保存在热带和温带环境的化石中的钙化软骨作为古DNA分析的新对象。

关键词：实验埋藏学，软骨细胞，骨细胞，死后自溶，冷冻相关细胞死亡，细胞和生物分子石化，古DNA研究对象

中图法分类号：Q915.865　　**文献标识码**：A　　**文章编号**：2096–9899(2023)02–0108–15

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