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Mechanobiological Implications of Articular Cartilage Crystals

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Abstract

Purpose of Review

Calcium crystals exist in both pathological and normal articular cartilage. The prevalence of these crystals dramatically increases with age, and crystals are typically found in osteoarthritic cartilage and synovial fluid. Relatively few studies have examined the effects of crystals on cartilage biomechanics or chondrocyte mechanotransduction. The purpose of this review is to describe how crystals could influence cartilage biomechanics and mechanotransduction in osteoarthritis (OA).

Recent Findings

Crystals are found in both loaded and unloaded regions of articular cartilage. Exogenous crystals, in combination with joint motion, result in substantial joint inflammation. Articular Cartilage Vesicles promote crystal formation, and these vesicles are found near the periphery of chondrocytes. Crystallographic studies report monoclinic symmetry for synthetic crystals, suggesting that crystals will have a large stiffness compared with the cartilage extracellular matrix, the pericellular matrix, or the chondrocyte. This stiffness imbalance may cause crystal-induced dysregulation of chondrocyte mechanotransduction promoting both aging and OA chondrocyte phenotypes.

Summary

Because of their high stiffness compared with cartilage matrix, crystals likely alter chondrocyte mechanotransduction, and high concentrations of crystals within cartilage may alter macroscale biomechanics. Future studies should focus on understanding the mechanical properties of joint crystals and developing methods to understand how crystals affect chondrocyte mechanotransduction.

Keywords

Osteoarthritis; crystal disease; cartilage; mechanotransduction; chondrocyte; aging

Word Count

2165 words

Introduction

Calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) are crystals associated with disease in human joints. There is a clear association between these calcium-containing crystals and osteoarthritis (OA), with most studies reporting calcium crystals in 40-70% of OA cases (1-3). Although studies agree that calcium-crystals are prevalent in a significant portion of OA cases, the clinical implications remain controversial. Some studies report that patients with chondrocalcinosis have an increased rate and severity of radiographic OA and greater disability if CPPD crystals are also present in the synovial fluid (4-6). Other studies find that OA patients with BCP crystals have more severe radiographic OA than those lacking crystals (1, 2, 7). OA patients with CPPD in their SF are also more likely to be symptomatic than asymptomatic (8). In summary, there is clear evidence that calcium crystal depositions contribute to the pathogenesis of OA, yet the mechanism(s) remain unclear.

While progress is being made in understanding crystal deposition, crystal prevalence, and treatment of crystal diseases, the implications of mechanical loads on crystal deposition and crystal-induced effects on chondrocyte mechanotransduction have yet to be resolved. The purpose of this review is to summarize current knowledge regarding the mechanobiological implications of crystals in articular cartilage.

Joints are responsible for transmitting mechanical forces that arise from skeletal function and during human movement. For example in the hip joint, contact forces can be more than 7 times body weight (9, 10). In a given joint these loads result in articular cartilage with both severe load-related deterioration and minimal deterioration associated with the absence of load (11). For almost three decades, we have known that chondrocyte metabolism is regulated at least in part by the mechanical loads transmitted through joints (12). Given that altered joint loading and dysregulated chondrocyte mechanotransduction are associated with OA (13, 14), the following questions arise: do mechanics play a role in crystal deposition? Do crystals alter chondrocyte mechanotransduction?

Cartilage is a soft material with spatially varying material properties. The extracellular matrix comprises about 90% of the tissue volume and has a stiffness of ~1000 kPa (*15, 16). The pericellular matrix surrounding chondrocytes is even softer with a stiffness of about 150 kPa (*15, 17), and the chondrocytes are the least stiff around 0.1 kPa (*18). We were unable to find published literature documenting the stiffness of CPP or BCP crystals. However, advances in crystallographic analysis of synthetic CPPD crystals find monoclinic symmetry (*19, *20) which

typically results in stiffness in the gigapascal range (21). Therefore, it is likely that the stiffness of crystals is orders of magnitude greater than the local tissue environment of articular cartilage. Because the relatively stiff crystals occur on the periphery of the relatively soft chondrocyte, aging-induced crystal formation may induce aberrant mechanotransduction in chondrocytes (Figure 1).

Articular cartilage vesicles are secreted by chondrocytes and form crystals.

Articular cartilage vesicles (ACVs) are extracellular, membrane-bound organelles found in both healthy and osteoarthritic articular cartilage. Although ACV functions remain largely unknown in healthy cartilage, recent work has identified ACVs containing RNA to suggest their potential use in intercellular communication of cell coding data (22). ACVs found in osteoarthritic articular cartilage have been identified as the source of calcium crystal formation through mineralization. This mineralization is regulated by the ratio of pyrophosphate (PPi) to phosphate (Pi) with increased PPi promoting CPPD formation (23, 24). CPPD crystals are formed from ACVs through ATP usage while basic calcium phosphate (BCP) crystals are formed predominately with beta-glycerophosphate (25, 26).

Understanding the how the balance between PPi and Pi affects crystal formation may lead to new strategies to reduce crystal prevalence and disease symptoms. PPi/Pi levels are regulated by ecto-enzyme metabolism of ATP on the surface of ACVs and through the ratio of NTPPPH (nucleotide triphosphate pyrophosphate hydrolase) to alkaline phosphatase enzyme activities (23, 27). Other work has shown PHOSPHO1 and IL-1 β as key players in altered PPi/Pi ratios. PHOSPHO1 is a phosphoethanolamine/phosphocholine phosphatase that draws phosphate into the ACV to begin mineralization (28). IL-1 β treated mesenchymal cells produce vesicles with less PPi and altered ENPP1 (ectonucleoside pyrophosphatohydrolase/phosphodiesterase 1) to alkaline phosphatase levels suggesting that inflammatory cytokines may trigger mineralization (29). *Yamakawa et al.* also noted an inflammatory cell response around CPPD crystals in tumor CPPD crystal disease patients (30). PC-1, a RDNP family gene expressed in chondrocytes, has been studied extensively by Terkeltaub *et al* who demonstrated its role in directly regulating ePPi (extracellular PPi) and indirectly regulating iPPi (intracellular PPi) through regulation of NTPPPH (31, 32).

Studies from *Yamakawa et al* show that hypertrophic or metaplastic chondrocytes from patients with CPPD crystal disease may be directly involved in crystal formation through

increased cartilage intermediate layer protein (CILP) expression in the pericellular matrix (30). Fuerst *et al* found a correlation between ACV mineralization and type X collagen, an indicator of cell hypertrophy, using histological analysis and digital-contact radiography in OA knees (*33, *34). These studies also identified a correlation between *in vivo* mineralization progression and the ability of chondrocytes to produce BCPs *in vitro*. *In vitro* chondrocyte hypertrophy led to mineralization of the extracellular matrix.

Osteoarthritis and cartilage mineralization

Healthy human articular cartilage is primarily composed of type II collagen and large proteoglycans, such as aggrecan. Jubeck *et al.* demonstrated that changes in cartilage extracellular matrix proteins consistent with OA matrix changes correlate to ACV mineralization (26). Including both type II collagen and large proteoglycans in the extracellular matrix of chondrocyte agarose gel constructs inhibited ACV mineralization. In contrast to healthy cartilage, OA cartilage has decreased type II collagen, fewer large proteoglycans, and increased type I collagen. The addition of type I collagen to chondrocyte agarose constructs containing type II collagen showed increased ACV mineralization suggesting a release of an inhibitory factor in type II collagen. Therefore, small quantities of type I collagen, decreases in type II collagen, and loss of large proteoglycans found pathologically in osteoarthritic cartilage may promote both CPPD and BCP crystal formation. These studies provide a link between osteoarthritis and cartilage mineralization.

The findings of Jubeck *et al* highlight the importance of extracellular matrix proteins in crystal formation. In a later study, this group compared collagen and collagen receptors on ACVs in human and porcine to explain the differences in mineralization behavior (35). They found isolated human ACVs to have increased type II collagen not found in porcine ACVs. Further, they suggest that isolated human ACVs result in poor mineralization largely due to residual type II collagen from sample preparation. Proteomic analysis of ACVs showed similarities and differences between normal and osteoarthritic cartilage with the differing proteins primarily consisting of extracellular matrix proteins in osteoarthritic cartilage (36). These studies emphasize the importance of tissue type, species, and sample preparation when studying mineralization due to changes in extracellular matrix proteins.

It remains unknown whether collagen is bound to ACVs through receptors or specific structural proteins. However, the presence of collagen could anchor the ACV onto the ECM or

PCM. Alternatively, the collagen profile of an ACV may identify the composition of the parent cell and/or signal matrix composition changes to the ACV (35). This could provide a mechanism for transmitting extracellular mechanical loading to chondrocytes for mechanotransduction.

Joint Crystals are Associated with Disease

Given that only some of the articular cartilage in a joint is load-bearing, early studies attempted to elucidate the spatial distribution of crystals in cartilage to determine if there's an association between crystal location and mechanical stress. In 1983, cuboid crystals were found almost exclusively in the superficial zone of structurally normal femoral head cartilage (37). In 1990, Stockwell *et al* found that crystal density was highest 0-50 nm from the articular surface compared to 50-500 nm from the articular surface regardless of cell proximity (38). However, crystal content was higher in regions surrounding cell debris. A follow-up study focused on the superficial zone and found that a band of microcrystals existed 10-20 um below the articular surface (*39). Additionally, crystals density was higher in superior (e.g. loaded) samples than inferior samples of femoral head cartilage. Given that superior cartilage regions experience higher mechanical stress (10, 11), this suggests that mechanical loading may be implicated in crystal formation. However, a more recent study revisited the spatial distribution of crystals in osteoarthritic articular cartilage with more sensitive methods and found that crystal content did not differ between superficial and deep layers, or between femoral compartments (40). In summary, crystals are found in both non-weight bearing and weight-bearing regions of articular cartilage and throughout the depth of the tissue.

Studies show a clear association between these calcium-containing crystals and osteoarthritis (OA), with most studies reporting calcium crystals in 40-70% of OA cases (1-3). Recent studies using advanced methods report lower co-existence of CPP crystals and OA, with co-existence in 22.3% of patients in Oliviero *et al* 2012, 17.7% in Ryu *et al* 2014, and 13.9% in Galozzi *et al* 2015 (Table 1). Interestingly, in Olivero *et al*, the analysis of OA SF revealed that age and inflammatory indices were significantly higher in SF positive for CPP crystals compared to those without crystals.

Another study investigated the prevalence of CPPD crystals in patients of Thai descent with OA (n = 100). This study found that 43% of patients had CPP crystals, 35% had

chondrocalcinosis, and 25% had both CPPD and chondrocalcinosis (*41). The average age at joint replacement was significantly higher for patients with crystals. This study also found no significant difference in function (e.g. standing from a sit, stair climbing, etc.) between OA patients with CPPD and OA patients without CPPD crystals. Thus, mechanical stress in OA patients in the presence of crystals did not inhibit their ability to accomplish basic daily activities. This finding may be limited to the patient population in this study, as other reports typically find worse symptoms, disability, and radiographic progression in CPPD disease than in OA (5, 8, 42). Furthermore, this finding may be specific to CPPD crystals given that BCP is also present in OA joints and associated with rapid progression of radiographic OA (1, 3, 7, *33, 43).

Chondrocyte Loading, Mechanical Stress, and Mechanotransduction

To our knowledge, only a single study directly tested the effect of mechanical stress on articular cartilage crystals. Given that clinicians prescribe rest to minimize the mechanical stress on joints to manage acute arthritis, Aguedo *et al* studied the effects of joint motion on crystal-induced articular inflammation (*44). Canine knee joints were injected with monosodium urate crystals and exercised with 90 degrees of flexion-extension for various durations. Exercise increased the inflammatory response in proportion to the duration of joint motion. Higher leukocyte counts, increased polymorphonuclear leukocyte infiltration, larger effusions, and increased clearance of intravenously injected isotopes from synovial vessels evinced this increased inflammatory response. In contrast, control joints not injected with crystals were grossly normal, with the exception of dilated vessels in the exercised joints. Thus, joint loading and associated mechanical stress can worsen the negative biological response to crystals in joints (Figure 1).

Articular chondrocytes are subject to nearly continual mechanical loading. Cyclical load-bearing is important for joint health as demonstrated by the marked hindlimb glycosaminoglycan loss that occurs following tail suspension in rats (45). Matrix metabolism is substantially altered by dynamic compression, but the balance between matrix synthesis and degradation is unclear as demonstrated by findings of both induced matrix transcription and synthesis (46, 47) and transcription and increased activity of the catabolic enzymes MMP-3,-9,-13 and ADAMTS-4 and -5 (48). Dynamic compression can induce phosphorylation of multiple enzymes including MAPK and SEK (49, 50), Akt (51), Erk -1 and -2 (52-54), and Rho kinase (55). These results originate from varying *in vitro* models. However, taken together some trends emerge: low-magnitude cyclical loading usually results in biological changes promoting matrix synthesis, and high

magnitude cyclical or static loading typically yields changes that result in matrix catabolism. Future studies may examine the both role of crystals in chondrocyte mechanotransduction and the role of mechanical loading in crystal deposition.

Conclusion

Calcium pyrophosphate dehydrate (CPPD) and basic calcium phosphate (BCP) are crystals that occur in articular cartilage and synovial fluid, and are associated with joint diseases such as OA. These crystals are formed from articular cartilage vesicles (ACVs) that are secreted from chondrocytes. CPPD and BCP crystal deposition occurs in both load-bearing and non-contacting areas of articular cartilage. The dramatic difference between the crystal stiffness and the stiffness of other cartilage components (ECM, PCM, and chondrocytes) may provide a mechanism by which crystals alter chondrocyte mechanotransduction (Figure 1.) Further research is required to test this hypothesis, but it is supported by early observations that the combination of joint loading and exogenous crystals result in severe inflammation (*44).

Key Points

- When joints contain crystals, joint motion results in inflammation.
- CPPD and BCP crystal deposition occurs in both contacting and non-contacting areas of articular cartilage.
- Crystals are formed from articular cartilage vesicles secreted by chondrocytes.
- The dramatic difference between the stiffness of the crystals and the stiffness of cartilage components (ECM, PCM, and chondrocytes) may provide a mechanism for crystals to alter chondrocyte mechanotransduction.

Acknowledgements

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Figure and Table Captions

Table 1 (Original Table) Reported prevalence of crystals in populations of osteoarthritis (OA) patients

Figure 1 (Original Figure) Conceptual model of how the presence of BCP and CPPD crystals may alter mechanotransduction in the aging chondrocyte. Crystal prevalence increases with aging, and crystals are formed from Articular Cartilage Vesicles on the chondrocyte periphery. In OA, the stiffness of the extracellular matrix (ECM) and pericellular matrix (PCM) decreases. Because crystals are substantially stiffer, they may promote dysregulated chondrocyte mechanotransduction in OA.

Figures and Tables

Table 1 Reported prevalence of crystals in populations of osteoarthritis (OA) patients

Study	Sample population	CPPD crystals [%]	BCP crystals [%]	CPPD and BCP Crystals [%]
Gibilisco <i>et al</i> 1985	SF from OA knee joint effusions	21%	30%	48%
Derfus <i>et al</i> 2002	SF from pre-operative OA knee joints			60% of SF had CPPD and/or BCP crystals
Nalbant <i>et al</i> 2003	SF from OA knee joint effusions	21%	47%	16%
Viriyavejkul <i>et al</i> 2006	SF from OA knee joint effusions prior to total knee replacement surgery	43%	NA	NA
Oliviero <i>et al</i> 2012	SF collected from outpatients undergoing arthrocentesis for non-traumatic joint effusions (positive for OA)	22.30%	NA	NA
Ryu <i>et al</i> 2014	Cadaveric knees with grade 3 cartilage degeneration of the femoro-tibial joint (OA-positive)	17.70%	NA	NA
Galozzi <i>et al</i> 2015	SF aspirated from wrist and finger joints (positive for OA)	13.90%	NA	NA

Figure 1 and Caption

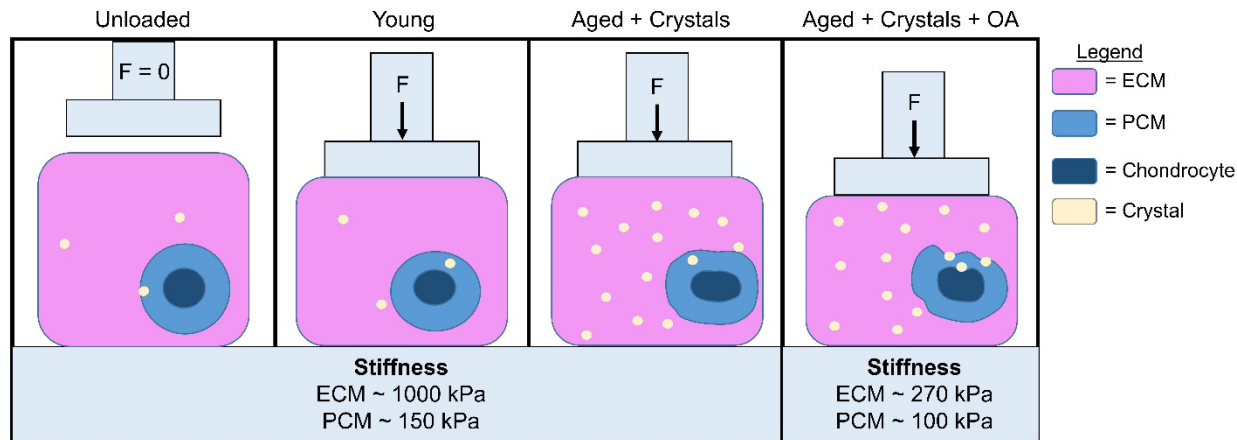


Figure 1: Conceptual model of how the presence of BCP and CPPD crystals may alter mechanotransduction in the aging chondrocyte. Crystal prevalence increases with aging, and crystals are formed from Articular Cartilage Vesicles on the chondrocyte periphery. In OA, the stiffness of the extracellular matrix (ECM) and pericellular matrix (PCM) decreases. Because crystals are substantially stiffer, they may promote dysregulated chondrocyte mechanotransduction in OA.