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Mechanoadaptation: articular cartilage through thick and thin

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Abstract:

The articular cartilage is exquisitely sensitive to mechanical load. Its structure is largely defined by the mechanical environment and destruction in osteoarthritis is the pathophysiological consequence of abnormal mechanics. It is often overlooked that disuse of joints causes profound loss of volume in the articular cartilage, a clinical observation first described in polio patients and stroke victims. Through the 1980s, the results of studies exploiting experimental joint immobilisation supported this. Importantly, this substantial body of work was also the first to describe metabolic changes that resulted in decreased synthesis of matrix molecules, especially sulfated proteoglycans. The molecular mechanisms that underlie disuse atrophy are poorly

understood despite the identification of multiple mechanosensing mechanisms in cartilage. Moreover, there has been a tendency to equate cartilage loss with osteoarthritic degeneration. Here, we review the historic literature and clarify the structural, metabolic and clinical features that clearly distinguish cartilage loss due to disuse atrophy and those due to osteoarthritis. We speculate on the molecular sensing pathways in cartilage that may be responsible for cartilage mechanoadaptation.

Background:

The mechanosensitivity of musculoskeletal tissues is extremely well documented but for the most part this has focused on muscle and bone rather than articular cartilage (Ziaaldini *et al.*, 2017; Galea *et al.*, 2017; Uda *et al.*, 2017). Part of the reason for this lies in the fact that the cartilage is not easily visualised; its thickness is inferred from the gap between the ends of the bone (the joint space) on a plain radiograph. Cartilage has only relatively recently been directly visualised by MRI.

Articular cartilage is adapted to withstand mechanical stress.

Articular cartilage develops and operates under very high compressive and shear stresses throughout its lifetime, and has only modest purported reparative capability, particularly in the elderly. Cartilage is an extracellular matrix-rich tissue where only 5-10% of the tissue volume is cell (Hunziker *et al.*, 2002). The principle fibrillar component is type II collagen, forming staggered arrangements of triple helical molecules that are highly cross-linked and organised in such a way that they lie parallel with the surface layer superficially but perpendicular to it in the deep regions (Figure 1). The complex organisation of primary and secondary structures provides cartilage with high tensile strength and the orientation of fibres allows mechanical loads to be dissipated over the surface, so as to minimise impact on the focal 'loading-bearing' regions of the joint. The tissue holds water by means of fixed negative charges offered by the proteoglycan aggrecan, itself aggregated on hyaluronan chains in the tissue (Dudhia, 2005). Aggrecan has attachment sites for many chondroitin sulfate and keratan sulfate glycosaminoglycan (GAG) chains that lie within the territorial and inter-territorial parts of the matrix. The hydration allowed by the fixed negative charge of these chains provides the tissue with a swelling pressure enabling absorption of, and resistance to mechanical shock. The

chondrocyte is separated from the territorial matrix by a specialised pericellular matrix (PCM). Together, the chondrocyte and the PCM form the 'chondron'. Chondrons are themselves orientated relative to local mechanical strain, exhibiting a polarised morphology that varies with tissue depth (Poole, 1997).

Loss of proteoglycans by cleavage of the core molecule by aggrecanases occurs in osteoarthritis and is regarded as a key pathological feature of disease (Glasson *et al.*, 2005). Tissue catabolism is also mediated by other members of the matrix metalloproteinase (MMP) family. MMP13, one of the mammalian collagenases, has a proven role in osteoarthritis (Mitchell *et al.*, 1996), whilst MMP3 (stromelysin) doesn't appear to drive disease and may be more important in normal tissue turnover (Clements *et al.*, 2003; Troeberg & Nagase, 2012).

Articular chondrocytes are highly mechanosensitive.

For articular cartilage to be able to respond to its mechanical environment, it must be able to sense a change in the magnitude and type of mechanical load and respond to this change. Historically, the chondrocyte was regarded as a largely quiescent cell, one that rarely proliferated and was responsible for slow turnover of the matrix. Early studies by Mankin showed that proteoglycan synthesis was moderately active (with a half life of around 8 days) (Mankin & Lippiello, 1969), and synthetic activity increased substantially following injury (Meachim, n.d.).

Multiple mechanisms by which chondrocytes sense mechanical load have been identified. Cellular mechanosensors include cell surface integrins, stretch and cation-sensitive ion channels (Ross *et al.*, 2013; O'Conor *et al.*, 2014; Lee *et al.*, 2014), see also review (Drexler *et al.*, 2014). The chondrocyte primary cilium has also been implicated in mechanotransduction, as it has in many other cell types (Wann *et al.*, 2012). Beyond the chondrocyte another important mechanism is release of sequestered heparan sulfate-bound molecules of the pericellular matrix (PCM) in response to compression or cutting injury (Vincent *et al.*, 2002; 2004; Vincent, 2013) (Figure 2). The best described of these is FGF2, which is chondroprotective *in vivo* in models of osteoarthritis (Chia *et al.*, 2009). Other molecules include connective tissue growth factor

(CTGF) which is covalently bound to latent TGFb. Release of the complex upon cutting injury results in activation of TGFb at the cell surface in a CTGF-dependent manner (Vincent, manuscript in revision). Activation of a number of intracellular signalling pathways occurs rapidly upon injury including TGFb-activated kinase 1 (TAK1), b-catenin, Src, Smads, mitogen activated kinases (ERK, p38 and JNK) and NFkB (Vincent *et al.*, 2002; Clements *et al.*, 2003; Gruber *et al.*, 2004; Dell'Accio *et al.*, 2006; 2008; Clements *et al.*, 2011; Troeberg & Nagase, 2012; Watt *et al.*, 2013; Ismail *et al.*, 2017). The receptor proximal mechanism of activation for most of these remains unknown.

Interestingly, both experimental data and in silico models show that the forces experienced by an individual chondrocyte are largely the same irrespective of the mass of the animal (Simon, 1970). This is most likely explained by 'isometric' scaling of the skeleton, where the joint surface area increases in proportion to the mass of the animal. Cartilage thickness does increase with animal size, but exhibits allometric rather than isometric scaling. Indeed, it has been suggested that there is an optimal maximum thickness of cartilage, beyond which perfusion of the chondrocytes deep in the tissue would be compromised (Malda *et al.*, 2013). One might thus hypothesise the existence of an evolutionary 'mechanostat' for chondrocytes that has been tuned to an optimal mechanical input. The mechanical input itself, being related to the properties (stiffness) of the surrounding matrix.

Evidence for disuse cartilage atrophy in humans

In 1974, Winford Pool reviewed 200 cases of flaccid paralysis of the lower extremeties. 25 cases had demonstrable narrowing of the hip joint space by plain radiograph of at least 50% (Pool, 1974). His conclusions at the time were that flaccid paralysis caused significant reduction of all types of load on the joint (compared with spastic paralysis where there is increased static load by action of muscle). He proposed that this reduced the nutrient supply to the cartilage, which is dependent upon pumping action of load-bearing activity. His findings were supported by those of Anderson and Breidahl who performed a similar study in 71 patients with either flaccid or spastic paralysis due to spinal cord damage. Their results confirmed the strong association between paralysis and joint space narrowing at the hip which was more common in flaccid rather than spastic paralysis (Anderson & Breidahl, 1981). Similar results were obtained when

lower limb amputees were examined. Importantly, in this paper they also comment that features of osteoarthritis were never seen on the amputated side (Benichou & Wirotius, 1982). This is one of the earliest clarifications regarding cartilage atrophy and OA. Despite both conditions leading to thinning of articular cartilage, OA develops as a result of increased mechanical load whereas atrophy results from reduced load.

The subsequent development in the early 1990s of non-invasive methodologies to assess the volume and proteoglycan content of articular cartilage by MRI allowed much more sensitive assessments of change with disuse and disease (Recht *et al.*, 1993; Haubner *et al.*, 1997; Lösch *et al.*, 1997; Eckstein *et al.*, 1997). A small number of cross-sectional and longitudinal studies have been carried out in spinal cord injury patients since. Cartilage volume loss was seen to decrease by around 6% in the first 6 months from baseline (baseline was on average 4 weeks post spinal injury), resulting in a loss of around 25% of the medial tibial thickness by 24 months after injury when compared with normal controls (Vanwanseele *et al.*, 2002; 2003). In individuals who are temporarily immobilised, through ankle fracture, a 6.6% loss of cartilage thickness was measured in the medial tibia over 7 weeks following fracture. Sensitivity of these techniques continues to increase and it is now possible to detect very small strain levels (around 3%) in the tissue that occur on normal weight-bearing activity (Eckstein *et al.*, 2000; Sutter *et al.*, 2015). No human study to our knowledge has demonstrated recovery of cartilage volume after remobilisation but importantly, moderate programmed exercise was shown to have a positive effect on increasing cartilage GAG content (assessed by dGEMRIC) in individuals at risk of OA due to previous meniscal injury (Roos & Dahlberg, 2005).

Disuse atrophy in large animal models

The greatest body of literature on cartilage atrophy stems from *in vivo* studies, mainly carried out in large animals (dog and rabbit). These studies have done much to provide definitive evidence for the importance of mechanics to joint homeostasis and disuse atrophy, greatly enhancing our understanding of the metabolic processes that lead to the condition. In the late 1970s Palmoski et al produced a series of interesting studies in which they immobilised healthy adult dogs using a light cast that fixed the knee joint at 90^{o}. The cast was strapped to the trunk preventing weight bearing or active flexion/extension. These studies revealed that 6 days of

immobilisation was sufficient to observe a reduction in proteoglycan staining in the tissue and by 8 weeks there was a 30-50% reduction in cartilage thickness with almost complete loss of proteoglycan. Although the authors didn't describe whether the changes occurred in the calcified or non-calcified cartilage, others have suggested that the calcified cartilage layer (below the tidemark) is more sensitive to atrophic changes (Kiviranta *et al.*, 1987). Interestingly, despite loss of proteoglycan there was an increase in the water content in the tissue, a feature that was confirmed by other similar studies (Palmoski *et al.*, 1979; Behrens *et al.*, 1989), and a change in the proteoglycan produced by the tissue such that it failed to aggregate with hyaluronan *in vitro*. This aggregation defect suggests a change in the GAG rather than the hyaluronan itself, although a reduction in hyaluronan tissue content has also been described with disuse (Haapala *et al.*, 1996). Hyaluronan synthesis itself was later shown to be mechanosensitive in the synovial joint (Ingram *et al.*, 2009). The hyaluronan aggregation defect was reversed upon voluntary remobilisation (Palmoski *et al.*, 1979) and may be related to restoration of normal hyaluronan synthesis (Müller *et al.*, 1994). Recovery from atrophy has been documented consistently in other studies, although is affected by the severity of atrophy attained and may be incomplete when immature joints are immobilised (Palmoski & Brandt, 1981; Behrens *et al.*, 1989; Kiviranta *et al.*, 1992).

In a follow up study by Palmoski et al, forced treadmill exercise prevented recovery of the tissue after immobilisation. Newly synthesised proteoglycans measured by ^{35}S incorporation demonstrated that there was a decrease in proteoglycan synthesis upon disuse, consistent with later studies examining aggrecan gene expression (Djurasovic *et al.*, 1998). Proteoglycan synthesis increased upon remobilisation in both the free and forced remobilised groups. The authors concluded that during forced remobilisation there must also be increased proteolytic activity to account for why proteoglycan failed to recover despite increased synthesis (Palmoski & Brandt, 1981). Whether increased proteolysis contributes to atrophic changes in the cartilage has been an area of speculation but to date there is little evidence to support this despite some efforts (Grumbles *et al.*, 1995; Haapala *et al.*, 2001).

The focus on proteoglycan metabolism in these studies is justified. There is significantly less information available to suggest a change in the collagenous matrix in atrophic cartilage apart from some modest reduction in collagen crosslinking which recovers after remobilisation

(Haapala *et al.*, 1999). This would be consistent with the known stability of fibrillar collagens and evidence for minimal turnover in human cartilage over the lifetime (Heinemeier *et al.*, 2016). The biomechanical consequences of matrix imbalance in atrophic tissue have been studied, ex vivo, by mechanical testing. These studies collectively show some site specific changes in shear and compressive properties of the cartilage, although show some inconsistencies (Jurvelin *et al.*, 1986*b*; Leroux *et al.*, 2001).

Disuse atrophy in small animal models

Generally speaking all types of joint immobilisation lead to atrophy although differences in how immobilisation is achieved, e.g. by rigid external fixation or cast immobilisation, and at which sites the effects are measured, does affect the type of outcome measures (Säämänen *et al.*, 1990) and magnitude of change (Behrens *et al.*, 1989). Two studies performed in the last decade have studied immobilisation in rodents. In the first, immobilisation was achieved by spinal cord injury in which the animals drag the affected limb. The study revealed changes in the thickness of cartilage across different regions of the joint at baseline with predicted weight-bearing regions having higher recorded thickness. Upon spinal cord injury a 30% decrease in cartilage thickness was measured in off-loaded areas (Moriyama *et al.*, 2008). The second study compared rigid (by externally fixed kirshner wires) and 'tail suspension' immobilisation in mice. Both led to a reduction in cartilage thickness, largely due to a decrease in the calcified cartilage, and were associated with loss of proteoglycan in the non-calcified layer particularly. Atrophy was slightly enhanced in the rigid fixation group, presumably because the joint was not subjected to movements associated with muscle activity (which are present during tail suspension) (Nomura *et al.*, 2017).

Increased thickness of cartilage upon exercise

If disuse leads to cartilage thinning then it follows that exercise training should result in thicker cartilage, so called cartilage hypertrophy. This has been shown in a number of large animal studies and is reflected by changes in the same biochemical metrics as measured in atrophy. Increased synthesis of the guanidine-extractable proteoglycan was documented in exercised rabbits (Tammi *et al.*, 1983; Säämänen *et al.*, 1988) and in dogs, there was an increase in extractable GAGs that were less able to aggregate with hyaluronan in vitro. These GAGs also had

increased ratios of chondroitin-6 to chondroitin-4 sulfate (Tammi *et al.*, 1983; Säämänen *et al.*, 1989). 4km of treadmill running a day for 15 weeks in dogs was associated with an increase in cartilage thickness (11% in loaded areas) and mechanical stiffness (6%) (Jurvelin *et al.*, 1986*a*). Treadmill training for up to 40 weeks at 5h/week, was associated with an increase in the non-calcified cartilage thickness by 19-23% and glycosaminoglycans by 28% (Kiviranta *et al.*, 1988).

Exercise dosage has been examined in rats running on a treadmill. Speeds of 15meters/minute with no incline, and 19 meters/minute with a 5^{o} incline for 5h per week for 6 weeks were associated with increased cartilage thickness and proteoglycan synthesis compared with non-exercised control animals (Ni *et al.*, 2013). Similar to studies of atrophy there are site-specific changes in cartilage that reflect the type of exercise undertaken e.g. down-hill treadmill running (Hamann *et al.*, 2014).

In contrast to exercise-induced adaptation of muscle and bone in adult human joints, there is much less evidence for cartilage thickness adaptation. While associations exist between rates of cartilage accrual and physical activity during childhood (Jones *et al.*, 2003), studies comparing athletes and inactive cohorts found the variance in cartilage thickness was not clearly attributable to activity levels (Eckstein *et al.*, 2002). However, an association between exercise and joint size (cartilage surface area) did exist, consistent with the isometric relationship described for body mass (or by extrapolation, load) and surface area in other mammals (Malda *et al.*, 2013).

Molecular mechanisms underlying mechanoadaptation in cartilage

The evidence thus far points to cartilage being highly 'mechanoadaptable' in contrast to its somewhat 'inert' reputation in the post-developmental context. Early speculation suggested that this was due to altered nutrition of the cartilage due to its dependence on mechanical load to deliver nutrients and oxygen to the chondrocytes. However, the relatively recent appreciation that chondrocytes can sense their mechanical environment and respond differently to distinct mechanical cues, amplitudes and frequencies, suggests that these mechanosensing mechanisms will turn out to be responsible for driving mechanoadaptation. Although, to our knowledge, this

has not been directly addressed, careful scrutiny of the literature does provide us with potential clues as to the pathways that may be responsible. For instance, increased cartilage thickness has been described in mice that have been subjected to a transient burst of Wnt signalling (by tamoxifen induced transgene activation) (Yuasa *et al.*, 2009). In this case there was an immediate loss of proteoglycan preceding the 'rebound' hypertrophy of cartilage. Wnt signalling occurs rapidly upon mechanical injury of cartilage in vitro and a number of wnt ligands are matrix bound and present in cartilage (Dell'Accio *et al.*, 2006; 2008; Berendsen *et al.*, 2011). Wnts are also involved in joint cavitation during development, a process that is critically dependent upon mechanical force by associated muscle action (Kahn *et al.*, 2009; Shea *et al.*, 2015).

Vincent has also observed an increase in cartilage thickness associated with constitutive activation of Smad2 signaling suggesting that TGFb family members may drive hypertropic cartilage responses (Vincent, manuscript in revision). As TGFb is one of the growth factors present in the pericellular matrix and decreases in cartilage with age (Madej *et al.*, 2015; van Caam *et al.*, 2016), it is tempting to speculate that its release upon tissue compression could be relevant to reduced mechanoadaptation over the lifecourse, a process described in other skeletal tissues (Greig *et al.*, 2011; Brook *et al.*, 2016). Another molecule that is involving in chondrocyte mechanosensing is FGF2. FGF2 is an important mechanosensitive mediator of joint cavitation during development (Kavanagh *et al.*, 2006; Shea *et al.*, 2015) and is also released by cartilage compression (Vincent *et al.*, 2007). However, we found no evidence of a change in cartilage thickness in male or female naïve FGF2 knockout mice (Chia *et al.*, 2009). Activation of cell surface mechanoreceptors piezo 1 and Trpv4 in chondrocytes leads to anabolic responses *in vitro* and chondroprotection *in vivo.* Moreover, their activity is associated with proteoglycan synthesis, making these interesting molecular candidates for further investigation.

(Clark *et al.*, 2010; O'Conor *et al.*, 2014; Lee *et al.*, 2014; O'Conor *et al.*, 2016).

What is the relationship between atrophy and osteoarthritis?

While mechanoadaptation of cartilage has become a somewhat neglected area of study, interest in molecular pathogenesis of osteoarthritis has greatly increased. Cartilage loss in OA has completely different molecular and chemical drivers; matrix catabolism is highly protease-dependent but associated with a strong anabolic reaction suggesting an unsuccessful repair response. Increased mechanical load is a critical aetiological factor that is strongly supported by epidemiological studies (Brandt *et al.*, 2009), and by showing that regulation of proteases *in vivo* upon induction of disease (by surgical joint destabilisation) is abrogated by joint immobilisation (Burleigh *et al.*, 2012). OA is also distinguished from atrophy by loss of integrity of the tissue starting with changes at the articular surface, which spread throughout the tissue as the disease progresses and are accompanied by aberrant bone remodelling. From a molecular and structural point of view, the process of atrophy and OA couldn't be further apart. The only feature they share is thinning of the cartilage and therefore an apparent decrease in the joint space on plain radiograph.

But is there a relationship between atrophy and OA? And why does exercise sometimes result in thickening of the cartilage and sometimes OA? The answer likely relates back to the defined 'mechanostat' within the tissue. Vincent and Wann speculate that if mechanical stimuli exceed a given threshold, or fall outside certain parameters, they are perceived as injurious and trigger pathways associated with rapid matrix remodelling and attempted repair. TAK1 activation appears to be critical in this response. TAK1 is a pivotal upstream regulator of inflammatory signalling, is induced by cartilage trauma and drives inflammatory gene regulation and matrix proteolysis (Ismail *et al.*, 2015; 2016). The upstream activator of TAK1 remains elusive and does not appear to be mediated by a soluble factor (Ismail *et al.*, 2017). Exceeding the injury threshold could occur either when the mechanical load is increased, or when the load is moderate but the joint has lost it mechanoprotective mechanisms. Examples of the latter include loss of stability of the joint and weakening of the cartilage matrix by atrophy. This would provide a plausible explanation for why ad libitum remobilisation leads to recovery after disuse but forced remobilisation leads to OA-like changes in the cartilage (Palmoski & Brandt, 1981). Similarly in joints weakened by papain injection, moderate exercise leads to rapid degeneration of the tissue (Siebelt *et al.*, 2014). A systematic review of experimental daily exercise in animals, also suggests a non-linear dose response with medium loads appearing good for cartilage but high loads precipitating degeneration (Bricca *et al.*, 2017).

We presume that the 'injury' threshold changes as the cartilage mechanoadapts and will be different for each individual according to genetics, the mechanical integrity of the tissue, the amount of load usually experienced by the joint and the 'pre-tuned' sensing mechanisms of their chondrocytes. Nonetheless, it is highly likely that mechanical training (graded exercise rather than overnight athleticism) will better protect the cartilage from load-induced activation of matrix remodelling and subsequent OA. Aiming for thicker cartilage is probably a good start. Whether it is possible to optimise cartilage thickness during skeletal development to prevent OA later in life, remains an important unanswered question.

Conclusions

Mechanoadaptation in cartilage is rapid and reversible, and potentially of a similar scale to that seen in muscle and bone. Comprehensive studies of cartilage atrophy *in vivo* have been carried out although largely during the 'pre-molecular' era and prior to the discovery of direct chondrocyte mechanosensing mechanisms. Advances in newly available glycobiology and proteomic techniques, in combination with genetic modification in rodents, will add considerable value to future studies.

Cartilage atrophy is readily distinguished from osteoarthritis at the clinical, tissue and molecular level but cannot be discerned on a plain radiograph except by noting the absence of bone remodelling. As joint space narrowing is typically used to diagnose OA, it is important to consider atrophy as a differential diagnosis. Harnessing the molecules that drive mechanoadaption in articular cartilage may provide novel strategies to prevent or treat OA.

Cause	Mechanism	Reversible?
Osteoarthritis	Proteolytic degradation of matrix	No, except by regeneration
Atrophy	Reduced synthesis of proteoglycan; a	Yes, weeks - months

	mechanoadaptive response to reduced load	
Cartilage compression	Water extrusion from matrix due to compression	Yes, minutes – hours
'Pseudo thinning'	Migration of tidemark.	Unknown
Reduction in joint space	Meniscal extrusion	

Table 1 Causes of thinning of the articular cartilage

Clinical features	**Cartilage Atrophy**	**Osteoarthritis**
Joint space narrowing (JSN)	Yes	Yes
History of immobilisation	Yes	No, increased load
Pain	No	Yes
Synovial hypertrophy/inflammation	No	Yes
Bone changes	Local osteoporosis	Subchondral bone sclerosis; osteophytes

Table 2. Clinical distinguishing features between cartilage atrophy and osteoarthritis

Tissue characteristic	**Cartilage atrophy**	**Recovery with ad libitum remobilisation?**	**Osteoarthritis**

Proteoglycan synthesis	Decreased	Yes	Increased
Total tissue proteoglycan	Decreased	Yes	Decreased
Evidence of proteolysis?	No	NA	Yes, of proteoglycan and collagen
Proteoglycan aggregation defect	Yes	Yes	Probably not
Chondroitin-6 sulfate	Not changed	Reduced relative to chondroitin-4-sulfate (Säämänen *et al.*, 1990)	Increased (Ratcliffe *et al.*, 1993)
Water content	Increased	Yes	Increased early in disease
Collagen cross-linking	Decreased	Yes	NK
Appearance	Smooth, shiny, healthy	NA	Roughened, dull

Table 3. Biochemical changes in atrophic and osteoarthritic cartilage

Figure 1. Schematic of articular cartilage. Illustration of tertiary structure of fibrillar collagens in articular cartilage showing parallel arrangement in superficial layer and perpendicular orientation in deeper layers. Arrows show direction of mechanical load and how it is dissipated across the articulating surface.

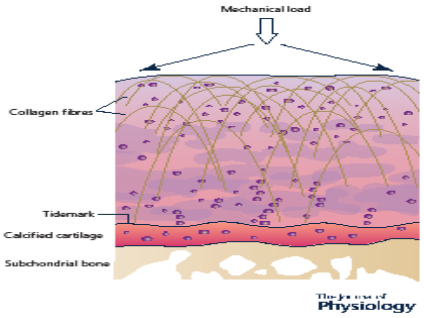
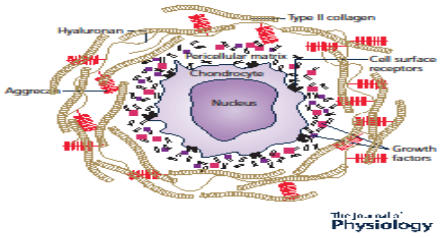


Figure 2. Schematic of a chondron. Illustration of the chondrocyte sitting within its pericellular matrix (PCM), together forming the chondron. Note absence of fibrillar collagens and aggrecan in the PCM, but abundance in the adjacent territorial matrix. Multiple heparan sulfate bound growth factors are bound within the PCM and released upon tissue compression or injury.



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Competing interests:

The authors can confirm that they have no competing interests to declare.