

Cartilage regeneration for treatment of osteoarthritis: a paradigm for nonsurgical intervention

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Abstract: Osteoarthritis (OA) is associated with articular cartilage abnormalities and affects people of older age: preventative or therapeutic treatment measures for OA and related articular cartilage disorders remain challenging. In this perspective review, we have integrated multiple biological, morphological, developmental, stem cell and homeostasis concepts of articular cartilage to develop a paradigm for cartilage regeneration. OA is conceptually defined as an injury of cartilage that initiates chondrocyte activation, expression of proteases and growth factor release from the matrix. This regenerative process results in the local activation of inflammatory response genes in cartilage without migration of inflammatory cells or angiogenesis. The end results are catabolic and anabolic responses, and it is the balance between these two outcomes that controls remodelling of the matrix and regeneration. A tantalizing clinical clue for cartilage regrowth in OA joints has been observed in surgically created joint distraction. We hypothesize that cartilage growth in these distracted joints may have a biological connection with the size of organs and regeneration. Therefore we propose a novel, practical and nonsurgical intervention to validate the role of distraction in cartilage regeneration in OA. The approach permits normal wake-up activity while during sleep; the index knee is subjected to distraction with a pull traction device. Comparison of follow-up magnetic resonance imaging (MRI) at 3 and 6 months of therapy to those taken before therapy will provide much-needed objective evidence for the use of this mode of therapy for OA. We suggest that the paradigm presented here merits investigation for treatment of OA in knee joints.

Keywords: articular cartilage, osteoarthritis, cartilage injury, cartilage repair, cartilage regeneration, cartilage organ size, osteoarthritis treatment, joint distraction, nonsurgical intervention

Introduction

It is estimated that about 10% of the world's population aged 60 years or older have significant clinical problems that could be attributed to osteoarthritis (OA) [Cooper *et al.* 2013]. The healthcare burden of OA on the society is enormous because this disease progressively affects the ageing population. Moreover, the ever changing population demographics further increase the cost of healthcare to society, and add pain and disability to a large segment of the population. Trauma, degenerative joint diseases, metabolic factors such as obesity and mechanical factors such as joint instability are among the common aetiological factors of OA [Felson, 2009]. Presently, there are no pharmacological agents for the prevention

or treatment of OA; the only medical option for OA involves pain management. In this context, cartilage tissue engineering has not held the high promises of therapy in OA [Huey *et al.* 2012] and the potential for cartilage regeneration through stem cell therapy has not yet fully materialized [Djouad *et al.* 2009; Johanson *et al.* 2012]. Accordingly, total joint replacement, though expensive, is considered a final option for relieving pain and regaining function in patients with OA [Gossec *et al.* 2011].

Articular cartilage is thought to have limited regeneration potential [Huey *et al.* 2012]. However, it has been observed that symptomatic pain relief and cartilage regeneration are possible

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in OA joints that have been surgically pulled apart or distracted for a prolonged periods of time [Wiegant *et al.* 2013]. This might have been a clue that cartilage regeneration is possible in OA joints. However, the mechanism(s) by which cartilage growth might occur in the distracted joint space are unknown. In this perspective, we analyse the biological aspects of cartilage development, including injury and growth, and present a conceptual context for cartilage regeneration in the distracted OA joints. Accordingly, we propose a novel paradigm for nonsurgical joint distraction treatment of OA.

Cartilage structure

Articular cartilage is a unique tissue between the ends of bones in the joints that helps in load bearing and lubrication. It is rich in extracellular matrix with scattered chondrocytes [Poole, 2005]. Adult cartilage has zonal architecture consisting of superficial, middle, and deep zones [Grogan *et al.* 2013]. These zones vary in matrix biochemical composition, cell density and morphology, cell metabolism and the pericellular matrix (PCM) [Wilusz *et al.* 2014]. The superficial zone extends ~10–20% of the full thickness of the articular cartilage. Chondrocytes in this zone are elongated, flattened, oriented parallel to the cartilage surface and are surrounded by densely packed collagen fibrils with low levels of aggrecan but higher concentrations of decorin and biglycan [Poole *et al.*

1996]. Superficial zone proteins homologous to proteoglycan 4 are encoded by the *Prg4* gene, which provides lubrication and a load-bearing surface for the articular cartilage [Ikegawa *et al.* 2000]. The middle or transitional zone comprises ~40–60% of the cartilage thickness. Chondrocytes in this middle zone are surrounded by randomly organized collagen type II fibrils with high concentrations of aggrecan, hyaluronic acid and dermatan sulfate (Figure 1). However, chondrocytes in the deep zone are ellipsoid, and these cells are stacked in cell columns surrounded by a specialized PCM and interspersed by a radially oriented collagen matrix [Poole, 2005; Wilusz *et al.* 2014]. Between the deep zone of cartilage and calcific cartilage in adults is a specialized amorphous material resembling a basement membrane called the tidemark (Figure 1).

Cartilage development

Development of articular cartilage and synovial joint structures is tightly synchronized [Koyama *et al.* 2008; Sandell, 2012; Iwamoto *et al.* 2013]. Initially at the prospective joint sites, expression of *Sox9/Col2/doublecortin* (DCX) in anlagen occurs in response to undefined upstream morphogenetic and determination mechanisms, and results in the expression of *Gdf5* in the interzone mesenchymal population [Decker *et al.* 2014]. This specific population of *Gdf5*-expressing joint progenitor interzone cells maintain DCX

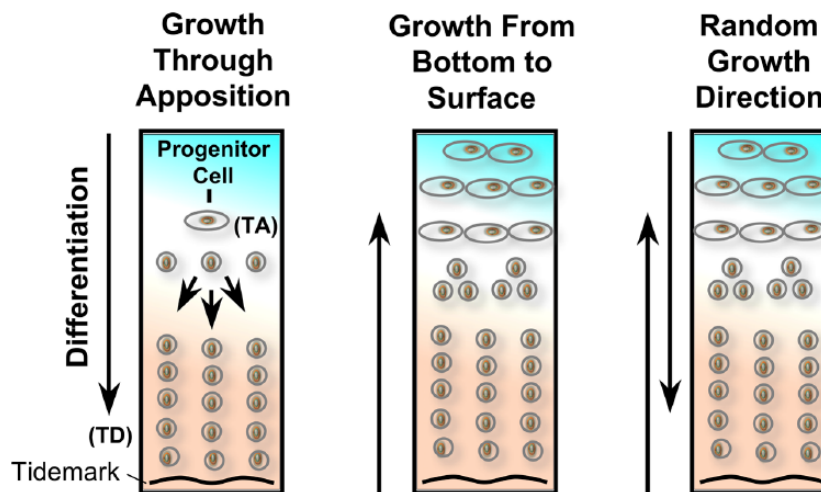


Figure 1. Hypothetical models of development of articular cartilage. Left panel shows growth through apposition. Middle panel suggests growth from bottom of cartilage attached to the bone towards cartilage surface in the joint cavity. Right hand panel suggests cartilage growth could be random from bottom and top of the cartilage.

Left side of figure is modified from Hayes *et al.* [2001]
TA, transit-amplifying; TD, terminal differentiation.

expression [Zhang *et al.* 2011]. In developing (digits) joints, Tgfr2 positive cells reach the interzone sites in spatiotemporal distribution [Li *et al.* 2013]. First, Tgfr2 positive cells are limited to the dorsal and ventral regions but undetected in the central region of the interzone [Spagnoli *et al.* 2007; Li *et al.* 2013]. Later on, anlagen-bound chondrocytes turn on the expression of matrilin-1. Gdf5 cells that subsequently express Sox9/Col2, and are positive for the expression of matrilin-1, differentiate into all other chondrocytes, while cells negative for matrilin-1 expression evolve into articular chondrocytes [Hyde *et al.* 2007]. This process involves specific action of regulatory genes including Erg and PTHrP [Iwamoto *et al.* 2007; Chen *et al.* 2008; Li *et al.* 2013]. Postnatally, Gdf5 and Tgfr2-positive and -negative interzone cells and their progeny give rise to multiple joint tissues including articular cartilage, intrajoint ligaments, synovial lining cells and other joint-specific structures such as the meniscus in the knee [Decker *et al.* 2014]. Moreover, Tgfr2 expressing cells constitute the slow cycling stem/progenitor cell population that are distributed with specific cell niches of joint such as in the groove of Ranvier [Li and Xie, 2005; Li *et al.* 2013].

Observations made through examining cartilage development in mice that are mutant for Noggin, Wnt4/Wnt9a, Tgfr2 or β -catenin revealed the presence of joint ablations and cartilage fusions. Similarly, mouse embryos deficient in the Tgfr2-activated kinase 1, heparin sulfate synthase Ext1 or Indian hedgehog (Ihh) also demonstrated joint defects [Gunnell *et al.* 2010; Mundy *et al.* 2011]. Furthermore, mouse embryo mutants for the master chondrogenic genes, Sox5 and Sox6, also display defects of limb skeletogenesis and joint development [Dy *et al.* 2010]. Similarly, the absence of the zinc finger transcription factors Osr1 and Osr2 limits the sustained expression of Gdf5, Wnt4 and Wnt9a in interzone cells leading to fusion of limb joints [Gao *et al.* 2011]. Also, ablation of C-Jun, which is an upstream regulator of Wnt9a, results in deranged Wnt signalling and lack of progression of joint formation [Kan and Tabin, 2013]. Collectively, these molecular regulators act at both local and distant levels and participate in the regulation of gene expression in interzone cells, and therefore control their cell fate and function. Adding to the ever expanding list of factors regulating cartilage development that were delineated above, low expression of chemokines and in particular MCP-5, compared

with growth plate chondrocytes, is also required for interzone cells and joint formation [Longobardi *et al.* 2012].

Developing joints next undergo cavitation to create the synovial cavity that is filled with lubricants containing the synovial fluid. It has been known for a long time that immobilization by experimental nerve damage inhibits cavity formation in the developing joints [Fell and Canti, 1934]. Joint movement stimulates the establishment of joint superficial cell phenotype and the production of essential lubricants such as hyaluronate and lubricin [Dowthwaite *et al.* 2003]. Recent studies using muscleless mouse embryo mutants have revealed that joint progenitor cell fate is not maintained in these embryos; neither Wnt/ β -catenin signalling is activated nor the joints have formed properly [Pazin *et al.* 2012]. Further studies revealed that there might be differential requirements for muscle-derived movements at joints such as the elbow joint compared with a more essential requirement for Tgfr2 signalling for knee joint formation [Pazin *et al.* 2012]. Nevertheless, data from ongoing research studies that are examining joint development indicate that, developmentally, articular chondrocytes are different from growth plate hypertrophic chondrocytes [Iwamoto *et al.* 2013]. Perhaps these studies may provide a key towards our understanding of cartilage regeneration and finding clues for treatment of cartilage injury.

Cartilage stem cells

After skeletal maturity, articular chondrocytes stop proliferating and are slow to replace. These cellular characteristics are also exhibited by stem cells [Blanpain *et al.* 2007]. When cultured, isolated articular chondrocytes form cobblestone sheets of overlapping cells and cartilage-like tissue. Chondrocytes exhibit characteristics of transformed cells, such as anchorage-independent growth, preference of a rounded cell shape and growth in nude mice [Benya and Shaffer, 1982; Dell'Accio, 2001]. These cell characteristics have been mostly associated with the cell biology of chondrocytes and need further reconsideration in the context of the features of cartilage stem cells.

Several independent research groups have demonstrated that cells isolated from the superficial zone of adult articular cartilage from various sources including human tissues have progenitor cell characteristics such as high colony formation

and expression of mesenchymal stem cell markers [Dowthwaite *et al.* 2004; Hattori *et al.* 2007]. Furthermore, after multiple passages, these cells express a chondrogenic phenotype and, in response to Tgfb, cells respond with enhanced synthesis of lubricin and cartilage specific matrix components [Dowthwaite *et al.* 2004; Hattori *et al.* 2007]. Furthermore, Tgfb and Wnt/ β -catenin signalling regulate proliferation and differentiation of these cells [Dowthwaite *et al.* 2004; Hattori *et al.* 2007].

Immunohistochemistry of normal cartilage sections shows large numbers of chondrocytes with staining for stem cell markers such as Notch-1, Stro-1 and VCAM-I throughout various zones [Grogan *et al.* 2009]. Stem cell surface markers such as CD44, CD151, CD105 and CD166 have also been demonstrated in chondrocytes [Alsalameh *et al.* 2004]. Interestingly, Hoechst 33342 dye exclusion, a feature of stem cell side population, is equally distributed in normal and OA chondrocytes [Grogan *et al.* 2009]. Furthermore, normal and osteoarthritic articular cartilage contains high number of CD105 and CD166 cells. In particular, the cell population containing CD166-positive cells have strong chondrogenic potential and were exclusively located in the superficial and middle zone [Pretzel *et al.* 2011].

Studies have also demonstrated that the synovium and infrapatellar fat pad contain mesenchymal stem cells, and it has been suggested that these progenitor cells could be utilized for cell-based tissue engineering and treatment of cartilage regeneration [De Bari *et al.* 2001; Drago *et al.* 2003; Futami *et al.* 2012]. Mesenchymal stem cells have also been found in the synovial fluid of normal knee joints and their number increases in synovial fluid of diseased joints [Jones *et al.* 2008; Morito *et al.* 2008]. Karlsson and colleagues studied BrdU labelling of adult rabbit knee joints and showed that cell labelling in the perichondrial groove of Ranvier is indicative for progenitor cells [Karlsson *et al.* 2009]. Moreover, these progenitor cells also stained for stem cell markers including Stro-1, Jagged1 and BMP1a [Karlsson *et al.* 2009]. As described above, Tgfb2 expressing cells contribute to joint development and populations of these slow cycling stem/progenitor cells are present in the perichondrial groove of Ranvier, synovium, articular cartilage superficial layer and tendon sites of joints. These authors suggested that this might be a specific site for a stem cell

niche in the joint that could have significant roles in repair and regeneration [Li *et al.* 2013]. However, given these observations, the progenitor cells in perichondrial groove of Ranvier will have an anatomical and physical barrier to traverse to the site of injury in the cartilage that usually occurs where the joint is subjected to repeated traumas – away from groove of Ranvier. However, it is still possible that progenitor cells of groove of Ranvier could be involved in increasing the circumference of the developing adult articular cartilage.

Bone marrow is rich source of hematopoietic and mesenchymal stem cells [Li and Xie, 2005]. Based on this concept, procedures for marrow stimulation including transcortical Pridie drilling, abrasion arthroplasty and microfracture rely on mesenchymal stem cells and growth factors coming from the bone marrow and have been advocated for cartilage regeneration [Schindler, 2011]. Koelling and colleagues observed that chondrogenic progenitor cells migrate to the damaged articular cartilage from the bone marrow through the subchondral bone [Koelling *et al.* 2009].

Collectively, it is unclear if there is a special niche within the confines of the articular cartilage that might support stem cell regeneration. Differentiated chondrocytes showing stem cell markers indicate the transit-amplifying nature of these cells but their significance in cartilage regeneration is still unclear [Blanpain *et al.* 2007; Doupe *et al.* 2012; Tata *et al.* 2013].

Cartilage maintenance

Homeostasis of the articular cartilage is maintained by the right balance between aggrecan and collagen contents to provide a physiochemical structure for compressive and tensile strength for joint load and mobility [Poole, 2005]. Aggrecan molecules are noncovalently associated with collagen fibrils and minor conditions such as immobility can cause its loss from the cartilage [Vertel, 1995]. However, chondrocytes rapidly replenish aggrecan. On the other hand, human collagen type II in cartilage matrix is a long-lived protein, with an estimated halflife of >117 years [Verzijl *et al.* 2000]. Thus, its breakdown, synthesis and maturation are complex and prolonged processes, and may be key limiting factors for regeneration of cartilage. Attrition of superficial chondrocytes by mechanical forces in the joint is likely replaced through its progenitor activity in a homeostatic

process. However, the middle and deep zones of cartilage show slow turnover of cells or matrix.

Cartilage injury and mechanisms of repair

Ideally, a cartilage injury should heal without a scar formation. Scar tissues within the joint would interfere with the smooth surface and would have deleterious mechanical properties. However, embryonic skin wounds heal without scar formation and are achieved by rapid re-epithelialization of wounds with little involvement from basal epidermal cells [Degen and Gourdie, 2012]. Embryonic wound edges are pulled together in a purse-string manner and small wounds in the gut epithelium also heal in this fashion [Degen and Gourdie, 2012; Polk and Frey, 2012]. In an *in vitro* wound model of bovine and human cartilage, it was observed that superficial zone chondrocytes rapidly proliferated, migrated and spread over the injured cartilage surface in a manner resembling re-epithelialization of wounds [Seol *et al.* 2012]. A cartilage injury does not involve fibrin clot formation, recruitment of inflammatory cells or angiogenesis [Martin, 1997]. However, cartilage tissue actively resists vascularization by producing anti-angiogenic components such as thrombospondin-1, chondromodulin-1, SPARC (secreted protein acidic and rich in cysteine), collagen type II derived N-terminal propeptide (PIIBNP), and the type XVIII derived endostatin [Patra and Sandell, 2012]. Furthermore, calcific cartilage and tidemarks are two anatomical barriers for the vascularization of articular cartilage. In OA, there are multiple depositions of tidemarks, as if to ward off vascularization from the subchondral bone. In support of this notion, injection of vascular growth factors in the joint cavity induces OA demonstrating its adverse effect in the joint [Ludin *et al.* 2013].

Murine and other animal models of OA usually involve cutting the medial meniscotibial ligament of knee joints. Microarray analysis of genes expressed in whole joints or microdissected tissues of joints, including articular cartilage, meniscus and epiphysis, which are obtained from these OA models, have advanced the molecular understanding of cartilage wound healing. A cartilage injury sets in motion a series of molecular events, which has parallels in other wounds, such as the expression of proteases and the release of growth factors [Martin, 1997; Wong *et al.* 2013]. In response to injury, there is a specific activation of inflammatory response genes in the injured

cartilage. The inflammatory gene responses that follow injured cartilage are not simply the same as those induced by agents such as interleukin 1 α (IL-1 α), and they are also independent of the effects of either gene activation or products from noncartilaginous tissues of the joints. In the injured cartilage, there is activation of the three MAP kinases JNK, p38 and extracellular-signal-regulated kinase (ERK), as well as Src kinases, phosphatidylinositol 3-kinase (PI3K) and NF- κ B [Chong *et al.* 2013; Watt *et al.* 2013]. In addition, hosts of other genes are also activated in injured cartilage, including those coding for cytokines and chemokines such as IL-1 α , IL-6 and CCL2 [Burleigh *et al.* 2012].

The injury response in cartilage can be categorized into catabolic and anabolic. Catabolic activity that is most pertinent to cartilage is the release of the aggrecan-degrading enzyme disintegrin, metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) and the collagenase matrix metalloproteinase 13 (MMP-13), which specifically degrade type II collagen. ADAMTS-5 is released early in response to injury and MMP-13 expression shows a delayed response [Burleigh *et al.* 2012]. In contrast, the anabolic activity includes the induction of chondroprotective genes such as tumor necrosis factor-inducible gene 6 protein (TSG-6), hyaluronan-binding anti-inflammatory molecule, tissue inhibitor of metalloproteinases-1 (TIMP-1) and activin A – a transforming growth factor beta (TGF- β) family member [Burleigh *et al.* 2012].

Several mechanisms control the signalling of inflammatory gene responses in a cartilage injury. Cartilage is a mechanosensitive tissue and perturbation of the matrix could signal the activation of chondrocyte surface receptors, such as that of integrins, calcium ion channels, primary cilium and discoidin domain receptors [Leong *et al.* 2011; Burleigh *et al.* 2012; Vincent, 2013]. When the integrity of the PCM is breached and subjected to cyclic compression, this results in the release of stored growth factors [Vincent, 2013]. The PCM is a specialized matrix that immediately surrounds chondrocytes and is rich in the nonfibrillar type VI collagen and the heparan sulfate proteoglycan perlecan, within which are sequestered various growth factors such as fibroblast growth factors (FGFs), connective tissue growth factor, insulin-like growth factor (IGF) and members of the TGF- β superfamily such as activin, bone morphogenetic protein (BMP) and their inhibitors [Vincent, 2013]. Of these, FGFs

have become significant, and FGF-2 and FGF-18 are the most studied. FGF-2, through its interaction with FGF receptor (FGFR)-1, promotes cartilage degradation and the engagement with FGFR-3 drives joint protection [Ellman *et al.* 2013]. In mouse models, FGF-2 delays cartilage degradation. Mice in which the gene for FGF-2 has been deleted develop accelerated OA spontaneously and following joint destabilization [Chong *et al.* 2013]. In the human cartilage, engagement of FGF-2/FGFR-1 acts as an antagonist by enhancing ADAMTS-5 and MMP-13 induction [Ellman *et al.* 2013]. However, engagement of FGF-18/FGFR-3 results in an anabolic effect in human articular chondrocytes, as reflected by increased chondrocyte viability and cell-associated matrix formation [Ellman *et al.* 2013]. In addition to the TGF family of proteins and growth factors, other factors such as Wnt signalling molecules are also liberated following cartilage injury [Dell'accio *et al.* 2008]. All of these growth factors are involved in the normal cartilage organogenesis and would therefore also play a role in OA cartilage.

Taken together, articular chondrocytes and the extracellular matrix participate in cartilage injury. The first task for the repair and regeneration of cartilage is chondrocyte activation and the release of cells from their locations in the matrix. This is achieved mainly by the release of ADAMTS-5 and MMP-13. Reactive oxygen radical production by activated chondrocytes may additionally facilitate the breakdown of the matrix [Tiku *et al.* 2000]. Once the damaged tissue is degraded, chondrocytes with progenitor and migratory abilities populate the injured site and begin the regenerative process [Morales, 2007]. In the superficial zone, the repair is rapidly achieved by superficial zone chondrocytes. However, the breakdown and regeneration of the middle zone to deeper layers of cartilage could be a slow process. Until recently, it was generally believed that cartilage regeneration is almost impossible in mammals. However, it has been observed in murine models that articular cartilage regeneration is heritable in that some murine strains are robust healers while other strains are nonhealers [Rai *et al.* 2013; Rai and Sandell, 2014].

There is a body of evidence that shows attempts of cartilage regeneration occurring in the middle and deep zones. For example, studies in OA have revealed that there is an initial increase in matrix synthesis and cell proliferation. Chondrocyte

clusters are formed in the middle and deep zones of cartilage [Brandt *et al.* 2008; Lotz *et al.* 2010]. However, in spite of these reparative processes, cartilage regrowth is generally not materialized in OA. The reasons for the failure of cartilage regrowth are unclear. Lessons from cartilage engineering have provided additional clues; for example, the lateral integration of neo-cartilage to adjacent cartilage is rarely observed [Huey *et al.* 2012]. Also, vertical fissures in OA cartilage have been known to fail to heal, indicting a failure of lateral integration of diseased OA cartilage tissue [Brandt *et al.* 2008].

Directional growth of articular cartilage

Superficial zone chondrocytes are thought to derive the appositional growth of cartilage (Figure 1) [Hayes *et al.* 2001]. This has enforced the notion that articular cartilage grows from the inside of a joint cavity towards the bone. This concept is at variance with the dynamics of development of other tissues, such as skin and mucosal linings of the gastrointestinal tract, which develop outwards from the corresponding underlying tissues. An embryonic stem cell population treated with Gdf5 and cyclopamine, a specific Hedgehog inhibitor, derived *in vitro* cartilage tissue, and showed zonal organization with columnar chondrocytes at the basal layer, a collagen type II-rich tidemark, and a distinct superficial layer [Craft *et al.* 2013]. This evidence indicates that cartilage can develop from the bottom up (Figure 1), suggesting that the mechanisms and dynamics of articular cartilage growth are still unresolved; in that regrowth may occur from top or bottom or may be random. Implications of these concepts also depend on the different levels of severity OA joints (Figure 2) and suggest that there might be a need of a layer of chondrocyte-rich matrix in ulcerated lesions that can be coaxed for regrowth.

Distraction of OA joints helps cartilage regeneration

Recent investigations of the role of joint distraction in OA therapy provide a clue for cartilage regeneration [Marijnissen *et al.* 2002; Wiegant *et al.* 2013]. The rationale for distracting joints is based on the leg-lengthening surgical procedure of Ilizarov. In this procedure, the broken ends of the bone are continually distracted. The bone is then filled with cartilaginous tissue, which is later converted into bone through the bone's healing process. The newly formed bone in the distracted

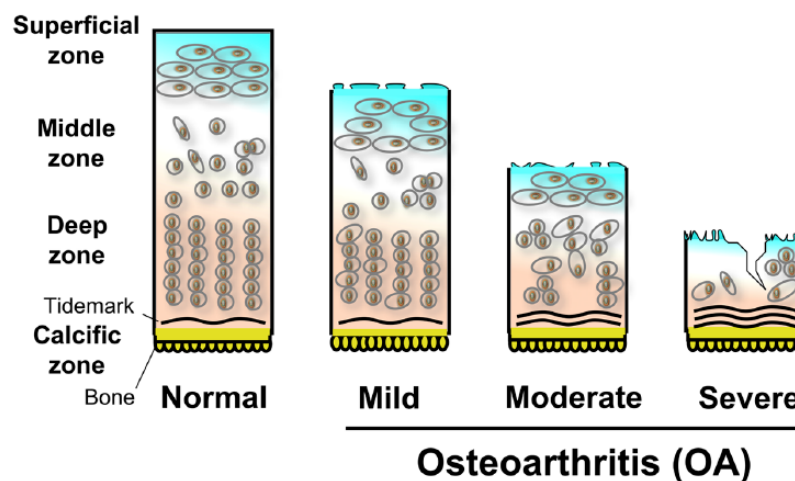


Figure 2. Diagrams representing normal and osteoarthritic (OA) cartilage. OA cartilage is depicted as mild, moderate and severe.

space provides solid bone tissue for lengthening the leg. In joint distraction, it is presumed that new cartilage tissue formed in a distracted space is pliable like normal cartilage and able to withstand compression and exhibit tensile properties. There is some corroborative evidence indicating that new cartilage tissue formed in distracted joints exhibits cartilage-associated properties [Wiegant *et al.* 2013]. The mechanism of cartilage regrowth in distracted joint space is not known. Moreover, factors that control tissue or organ dimensions in the body in general are poorly understood, except to some extent in certain pathological conditions [Zhao *et al.* 2011; Tumaneng *et al.* 2012]. Transcription co-activators such as Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are known to play a role in organ size control and regeneration, and it appears that these two processes are closely related. Also, these transcription factors have been identified as sensors and mediators of mechanotransduction, including in chondrocytes, suggesting that they play roles in cartilage regeneration [Dupont *et al.* 2011; Zhao *et al.* 2011; Zhong *et al.* 2013]. Another possibility is that negative pressure created in a distracted joint may accelerate cartilage wound healing. This concept is supported by the observations of the effects of negative pressure on the skin wound healing process [Orgill and Bayer, 2013; Schluck *et al.* 2013].

Although joint distraction by surgery provides a therapeutic opportunity for OA, it may not be an optimum or practical undertaking for a number

of reasons. The surgical procedure for distraction is a serious undertaking that involves a prolonged period of convalescence, with associated adverse effects such as pin tract infection. This procedure usually involves joint immobilization, which can lead to unfavourable effects and cause atrophy of cartilage. In addition, the lack of cyclic compression on the cartilage can eliminate any beneficial anti-inflammatory and anabolic effects of mechanotransduction on chondrocytes [Leong *et al.* 2011].

Monitoring cartilage degeneration and regeneration

The lack of consistently reliable tools to monitor cartilage degeneration and regeneration has hindered clinical studies in OA. Recent studies have indicated an advantage of magnetic resonance imaging (MRI) studies of joints [Guermazi *et al.* 2013]. An MRI examination can detect early abnormalities of cartilage, ligaments, menisci, synovial joints, bone and bone marrow. An MRI examination shows a three-dimensional visualization of cartilage and can provide quantitative, semi-quantitative and biochemical assessments of cartilage. Abnormal features in an MRI can predict rapid cartilage loss [Roemer *et al.* 2009, 2012]. These observations indicate that it is possible to monitor cartilage pathology by MRI in OA [Hunter *et al.* 2011]. Moreover, molecular assays, gene expression profiling and biomarkers of OA severity such as microRNAs [Beyer *et al.* 2015] could be used as secondary outcome measures to group responders and nonresponders

based on MRI studies for more qualitative measures and better understanding of the mechanisms of repair.

Paradigm of nonsurgical distraction therapy of OA

Considering the aforementioned observations, we suggest a study paradigm of cartilage regeneration in OA. Patients with OA of the knee would have MRI confirmation of their disease. According to MRI-defined qualitative, quantitative and biochemical assessment, cartilage degeneration may be graded as mild, moderate or severe (Figure 2). Mild disease is defined as being confined to the upper third of the cartilage; moderate disease extends up to two-thirds of the cartilage, while severe disease extends beyond these anatomical limits (Figure 2). Patients with mild or moderate cartilage degradation, with or without osteophytes or subchondral bone lesions with compartmental disease, will enter the clinical study protocol. Exclusion criteria would include a history of trauma, ligament or meniscal injury, obesity, joint instability or factors that could predispose to rapid cartilage degradation. Patients in the proposed pilot study will be allowed a normal wake-up activity of the index-knee joint using knee unloader braces to reduce stress in the diseased compartment and keep the opposing cartilage from causing mechanical friction [Lafeber *et al.* 2006]. During sleep, the index-knee joint will be subjected to a pull traction device to separate the bone ends of the knee joint and enhance negative intra-articular pressure. It should be noted that sleep-time traction would sufficiently distract the joint. An MRI study will be repeated at 3 and 6 months of therapy or later. In a blinded manner, the baseline MRI values and values obtained at the midpoint and endpoint of the study will be compared. The MRI technique will provide sophistication and precision in measuring cartilage and bone changes, bone marrow lesions, as well as the biochemical changes of cartilage. An advantage of the proposed study is that it is a non-invasive medical intervention and allows reasonable daily activity. In addition, it allows joint movements and mechanotransduction of chondrocytes without adverse effects of distraction on cartilage growth. Recently, patellofemoral braces have been shown to effectively reduce symptoms and bone marrow lesions in patients with patellofemoral OA [Callaghan *et al.* 2015]. Furthermore, studies of combination of subchondral drilling, joint motion and distraction promoted repair of

osteochondral defect in the weight bearing area in a rabbit model of cartilage injury, therefore suggesting that joint distraction may be advantageous for cartilage regeneration [Kajiwara *et al.* 2005]. Distraction of osteoarthritic ankles also helps in the remodelling of subchondral bone [Wiegant *et al.* 2013]. We believe that the paradigm presented here merits full investigation for the treatment of OA.

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Conflict of interest statement

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