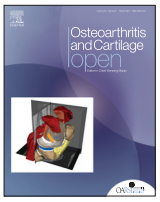
Osteoarthritis and Cartilage Open 4 (2022) 100306

Contents lists available at ScienceDirect

Osteoarthritis and Cartilage Open

journal homepage: www.elsevier.com/journals/osteoarthritis-and-cartilage-open/2665-9131



Experimental models to study osteoarthritis pain and develop therapeutics

Kanyakorn Riewruja [a,b], Meagan Makarczyk [a,c], Peter G. Alexander [a], Qi Gao [d], Stuart B. Goodman [d], Bruce A. Bunnell [e], Michael S. Gold [f,**], Hang Lin [a,c,*]

[a] Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Osteoarthritis and Musculoskeleton Research Unit, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, 10330, Thailand
[c] Department of Bioengineering, University of Pittsburgh Swanson School of Engineering, Pittsburgh, PA, USA
[d] Department of Orthopaedic Surgery, Stanford, CA, USA
[e] Department of Microbiology, Immunology, and Genetics, University of North Texas Health Science Center, Fort Worth, TX, USA
[f] Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

ARTICLE INFO

Keywords:
Osteoarthritis
Pain
Microphysiological system
Tissue chip
Nerve
Pain mediators

ABSTRACT

Pain is the predominant symptom of osteoarthritis (OA) that drives patients to seek medical care. Currently, there are no pharmacological treatments that can reverse or halt the progression of OA. Safe and efficacious medications for long-term management of OA pain are also unavailable. Understanding the mechanisms behind OA pain generation at onset and over time is critical for developing effective treatments. In this narrative review, we first summarize our current knowledge on the innervation of the knee joint, and then discuss the molecular mechanism(s) currently thought to underlie OA pain. In particular, we focus on the contribution of each joint component to the generation of pain. Next, the current experimental models for studying OA pain are summarized, and the methods to assess pain in rodents are presented. The potential application of emerging microphysiological systems in OA pain research is especially highlighted. Lastly, we discuss the current challenge in standardizing models and the selection of appropriate systems to address specific questions.

1. Introduction

Osteoarthritis (OA) is a disease of the whole joint that involves the pathological alteration of all components. There are currently more than 500 million OA patients worldwide [1], and as recently as 2019, OA was the 15th highest cause of years lived with disability (YLDs) worldwide, accounting for 2% of the total global YLDs [2]. OA may occur in all joints, including the hands, shoulders, hips, knees, and ankles. However, it is most common in knees, where symptomatic knee OA accounts for 17–35% of the population older than 50 years, and 88% of OA patients are older than 45 years [3]. Risk factors for OA include female menopause, obesity, history of joint injury, mechanical overuse, and abnormal joint alignment. Because advancing age is the most significant and common risk factor for knee OA, the incidence and impact of OA will increase dramatically in the face of an aging world population.

Pain is the predominant symptom of OA that causes patients to seek medical care and impairs their quality of life. Early OA pain symptoms are usually limited to affected joints and are intermittent depending on the intensity of daily activity. OA pain gradually becomes more severe, persistent, and unpredictable. Whether or not the same mechanism(s) underlie the pain associated with early and late phases of OA remains to be determined. However, the observation that non-steroidal anti-inflammatory drugs (NSAIDs) appear to lose antinociceptive efficacy with disease progression suggests additional mechanisms underlie OA pain in the later stages of the disease. Furthermore, pain-amplifying circuitry within the central nervous system is also likely to contribute to the pain associated with later stages of the disease. This suggestion is based on evidence that the presence of central sensitization in late-stage OA patients is similar to that observed in patients with chronic pain associated with other diseases [4].

Currently, the therapies for OA pain control have limited efficacy and severe side effects [5]. For example, NSAIDs are the initial drug of choice, either oral or topical use. However, these are associated with significant side effects on the gastrointestinal tract and other systems. Corticosteroid

* Corresponding author. Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
** Corresponding author.
E-mail addresses: riewruja@pitt.edu (K. Riewruja), mjm392@pitt.edu (M. Makarczyk), pea9@pitt.edu (P.G. Alexander), qigao7@stanford.edu (Q. Gao), goodbone@stanford.edu (S.B. Goodman), bruce.bunnell@unthsc.edu (B.A. Bunnell), msg22@pitt.edu (M.S. Gold), hal46@pitt.edu (H. Lin).

https://doi.org/10.1016/j.ocarto.2022.100306
Received 6 July 2022; Received in revised form 25 July 2022; Accepted 9 August 2022

intra-articular injections are also used in patients with severe pain but cannot be used repeatedly because of the potential for joint damage, and intra-articular hyaluronic acid injections are not routinely recommended. Opioids are the last line of defense for severe OA pain due to serious side effects, including increased risk of falling, constipation, development of opioid-use disorders (i.e., addiction), respiratory depression, and death. Joint replacement surgery currently remains the only therapeutic option for most in the late stages of the disease. However, this type of surgery is not recommended to young OA patients due to the finite lifespan of prosthesis [6]. Complications have also been reported, such as stiffness, thromboembolism, infection, and other anesthesia-related complications [7]. In addition, it fails to address chronic pain in ~20% of patients [8]. Therefore, there is an unmet clinical need to treat OA and associated pain.

The absence of more effective treatments for OA pain is clearly due to several factors, primarily rooted in our limited understanding of OA pathogenesis. While the available evidence suggests that both peripheral and central mechanisms contribute to OA pain [9], the efficacy of total knee replacement surgery suggests that peripheral mechanisms are primarily responsible for the majority of OA patients. Consequently, this review focuses on structural changes in the knee joint and the peripheral mechanism of OA pain signaling pathways. The involvement of the central nervous system and dorsal root ganglia (DRG) neurons in OA pain has recently been reviewed elsewhere [10], and is therefore not discussed here.

2. The knee joint – a highly innervated organ

The knee joint is an organ innervated by both sympathetic and sensory neurons. During healthy conditions, adult hyaline cartilage is aneural, which, however, can be innervated in the osteoarthritis [11]. In contrast to innervation of the skin, where neurons give rise to unmyelinated and thinly myelinated axons referred to as C- and Aδ-fibers, respectively, type IV and type III afferents, which are nociceptors, innervate the knee joint. Moreover, there is clearly a subpopulation of neurons with more heavily myelinated, namely Aβ- or type II fibers, which mostly encode stimuli in the non-noxious range [12]. Neurons typically considered to be the “pain fibers” innervating the knee are generally type III and type IV fibers. The response properties of the sensory neurons that innervate the knee have been most extensively characterized *in vivo* in the context of mechanical stimuli, where the nociceptive afferents appear to be most responsive to torque, a mode of mechanical insult most likely to damage the joint. However, studies suggest these neurons are also responsive to chemical and possibly thermal stimuli, suggesting that most of them would be classified as polymodal nociceptors [13].

Nociceptive afferents have been categorized by several properties in addition to axon conduction velocity. One of the more common properties has been whether the neuron expresses the neuropeptides substance P (SP) and, more commonly, calcitonin gene-related peptide (CGRP), where those with these neuropeptides are peptidergic and those without are non-peptidergic. More recent single-cell expression analysis suggests more subpopulations of putative nociceptive afferents [14]. However, because most of these single-cell analyses have been performed on neurons not identified by their target of innervation, the subpopulations identified are likely biased toward the most common subpopulations of neurons. Given that a recent analysis of neurons retrogradely labeled from the colon identified two populations not previously identified [14], it will be essential to perform comparative studies on the knee joint, where ideally it would be possible to differentially label the various components of the joint to determine whether there are subpopulations of sensory neurons specific to any of the knee joint components. All said, sensory neurons innervating the knee express both CGRP and SP. This is potentially important in the context of OA, given that these peptides are not only released from the central terminals of nociceptive afferents where they appear to contribute to the nociceptive signaling [15], but they are released in the periphery, where they appear to contribute to regulating cells involved in joint remodeling (i.e., osteoblasts and osteoclasts), and the immune response to tissue injury [16]. Consistent with this efferent role for the afferent innervation of the knee, there is evidence that joint damage in models of OA is exacerbated in the absence of peptidergic innervation of the joint [17].

Sympathetic nerve fibers were found in joint structures, including synovium, infrapatellar fat pad, and subchondral bone [18]. The effects of sympathetic neurotransmitters were demonstrated on bone and cartilage homeostasis and pain mechanisms in OA [19]. Available evidence suggests at least two mechanisms whereby sympathetic nerve fibers may contribute to the pain of OA. First, there is evidence that they can release inflammatory mediators such as prostaglandin E2. Second, they may release mediators, including norepinephrine that can act indirectly on cells such as macrophages or directly on nociceptive afferents [20]. Robust sprouting of postganglionic sympathetic nerve fibers along with sensory nerve fiber innervation was observed in the synovium of Freund's complete adjuvant mice arthritis model [21]. These changes may contribute to the pain associated with OA, which is supported by the observation that sympathectomy used to be a rescue treatment for patients with rheumatoid arthritis in the setting of chronic joint pain [22]. In addition, beta-2-adrenergic receptors antagonist alleviate pain sensitivity from high catecholamine levels and sympathetic responses in rat models of OA [23]. This effect was also found in human OA patients taking beta-blocker drugs, as they reported lower Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scores and required lower opioid treatment [24]. From a clinical perspective, persistent pain disorder due to abnormal sympathetic activity was called reflex sympathetic dystrophy (recently named complex regional pain syndrome [25]). However, in experimental pain-related OA models, it appears that the sympathetic nervous system is not directly involved.

3. Joint elements and their contribution to OA pain

Currently known mechanisms of OA pain locally in the knee joint are summarized in Table 1 and Fig. 1. The contribution of each joint element is discussed below.

3.1. Cartilage

In the absence of tissue injury, adult articular cartilage is an aneural and avascular structure. At the early stage of OA, cartilage surface fibrillation is often seen [40]. Next, chondrocytes undergo hyper-proliferation in response to matrix loss and form clusters. Other pathological changes observed in chondrocytes in the presence of OA include fibrosis, senescence, hypertrophy, and apoptosis [41]. Senescent chondrocytes isolated from OA cartilage have been shown to generate cytokines and chemokines that contribute to nociceptor sensitization and/or activation [42]. Furthermore, and consistent with a potential role for this tissue in OA-associated pain, the presence of innervation and vascularization has been documented in cartilage obtained from OA patients [43]. The increase in vascularization and innervation is associated with an upregulation in the expression of vascular endothelial growth factor (VEGF) and NGF [44]. Furthermore, there is evidence that chondrocytes from OA patients can be stimulated to release inflammatory mediators. For example, chondrocytes appear to express P2X3, and P2X2/3 receptors, receptors for ATP released from sympathetic postganglionic neurons and other cells, which when activated are associated with the release of inflammatory cytokines including TNF-α, IL-1β, IL-6, and CINC-1 [45].

3.2. Synovium

In a healthy joint, the synovial membrane consists of two layers. The outer layer is comprised mainly of collagen type I, lymphovascular channels, as well as nerve fibers. In contrast, the inner layer primarily

Table 1
Currently known OA pain mediators.

Potential mediators	Actions	References
Inflammatory cytokines	Pain upon movement was positively correlated with TNF-α, IL-6, and IL-8, and negatively correlated with IL-1β, while pain at rest was associated with high levels of TNF-α.	[26]
Chemokines	CCL12, a monocyte chemoattractant protein, is increasingly expressed in the synovial lining and synovial fluid of OA knees and following mechanical injury	[27]
	Manipulations of the CCL2/CCR2 axis in this model influence pain behaviors but not the structural changes in DMM model of OA	[28]
Nerve growth factor (NGF)	Intraarticular Anti-NGF monoclonal antibodies injection reduced local knee pain in MIA mouse OA model	[29]
Brain-derived neurotrophic factor (BDNF)	mRNA of BDNF and TrkB genes were upregulated in human OA synovium, and BDNF was present in OA synovial fluid. The sequestration of BDNF via TrkB FC chimeric acutely reversed OA pain behaviors, while intraarticular injection of BDNF further exacerbated them in MIA and MNX models.	[30]
Acid-sensing ion channels (ASIC)	An increase in ASIC3 in both the synovium and DRG was found in the MIA model of OA in the rat. Mechanical hyperalgesia is attenuated in genetically eliminated mouse models (i.e., ASIC3 knock-out) despite an increase of inflammatory mediators, namely IL-6, matrix metalloproteinase (MMP-3), and MMP-13.	[31]
Transient receptor potential (TRP) cation channels	Systemic administration of TRPV1 antagonists has been shown to attenuate hypersensitivity in MIA rat OA models but not in sham rats.	[32]
	TRPV1 agonist was able to polarize M1 macrophages to an M2 phenotype and reduce synovial inflammation through the Ca2+/calmodulin-dependent protein kinase II (CaMKII)/nuclear factor-erythroid 2-related factor 2 (Nrf2) signaling pathway	[33]
	Hypersensitivity associated with the MIA model of OA is attenuated in TRPA1 knock-out mice	[34]
	Systemic administration of the TRPA1 antagonist A-967079 attenuated the increases in spontaneous and evoked activity in the spinal cord dorsal horn neurons in the MIA model of OA	[35]
	There was an increase in TRPM8 activity in mouse DRG neurons retrogradely labeled from knee challenged with OA synovial fluid	[36]
	Mechanical hypersensitivity associated with the MIA model of OA is attenuated in TRPV4 knock-out rats	[37]
	Hypersensitivity was increased with TRPV4 agonists and decreased with TRPV4 antagonists that were administered intraarticularly in MIA model of OA	[38]
Voltage-Gated Sodium (Nav) channels	Selectively blocking Nav1.7 and Nav1.8 had efficacy in the rat MIA model of OA with both system and local administration of antagonists	[39]

(Acronyms: OA-osteoarthritis, MIA-monoiodoacetate-induced OA model, MNX-medial meniscal transection-induced OA model. DMM-destabilization of medial meniscus-induced OA model, TNF-α-tumor necrosis factor-α, IL-6-interleukin-6, and IL-8-interleukin-8, IL-1β-interleukin-1β, CCL2-Chemokine (C–C motif) ligand 2, CCR2- CC-chemokine receptor 2, TrkB-tropomyosin receptor kinase B, TRPV-transient receptor potential cation channel subfamily V member, TRPA-transient receptor potential ankyrin family, TRPM-transient receptor potential melastatin family).

contains two synoviocytes, macrophage and fibroblast-like cells [46]. Structural changes observed in the synovium associated with OA include hyperplasia, infiltration of mononuclear cells, activated T and B cells, macrophage clusters, and the production/release of pro-inflammatory cytokines [47]. The association between synovial inflammation (synovitis) and pain has been investigated. The infiltration of inflammatory cells like helper T-cells (Th), specifically Th1 CCR5+ cells, into synovium was significantly increased in the synovial membrane from patients who reported greater pain and correlated with functional disability [48]. Klein-Wieringa also demonstrated that activated helper T cells found in synovium membranes were associated with higher pain scores in OA patients [49]. Another study identified that macrophage-inhibitory factor in the synovial fluid, not in serum, positively correlated with WOMAC pain score [50]. In addition to the infiltrating immune cells that are capable of releasing mediators and likely contributing to the pain of OA, there is evidence that the native cells residing in the synovial tissues can also generate pain mediators. In particular, these cells have been shown to release BDNF, which, as noted previously, is a trophic factor implicated in nociceptive signaling [30].

Evidence also suggests the correlation between sensory innervation, indicated by CGRP expression, and inflammation in synovial tissue. In an *in vitro* study, Minarani and co-workers treated synovium tissue explants with TNF-α, IL-1β, or PGE2, resulting in an increased expression of CGRP [51]. In the MIA-induced OA model, Hoshino and co-workers showed the correlation of pain-related behavior and synovial hypercellularity, which proved to be faster and irreversible at a higher dose of MIA which showed a positive correlation of CGRP nerve fibers in the synovial tissue [52]. Bourassa et al. also described an increased density of sensory and sympathetic nerve fiber sprouting in the MIA-induced OA rat model, similar to the neuropathic model [53]. In agreement with the observations above, a study by Obeidat et al. showed dense innervation in lateral synovium and increased signal in deep later of medial synovium in the knee joint of mice undergoing DMM [54]. Also, from OA patients, synovium showed a positive correlation of COX2 and CGRP receptor expression [47]. Together, inflammation in the synovium appears to contribute to neuronal sprouting.

Lastly, the study of pain-associated genes from OA patient synovial biopsies showed that neuronal proteins were the top three genes that were differentially expressed in high pain patients, which were the Stress Responsive DNAJB4 Interacting Membrane Protein (SDIM1), Carboxypeptidase E (CPE), and Otoferlin (OTOF). SDIM1 and CPE play a role in the cellular stress response. OTOF is a transmembrane protein that can modulate the GABAergic activity in the GAD65-dependent manner in neuronal and non-neuronal cells [55].

3.3. Infrapatellar fat pad

The intrapatellar fat pad (IPFP) is an intracapsular but extrasynovial tissue localized anteriorly distal to the patella. IPFP from OA patients showed structural changes, including increased inflammatory infiltration, vascularization, and thickened interlobular septa [56]. Currently, there is a disagreement as to whether the fat pad volume is associated with pain levels reported in OA. However, other evidence suggests the contribution of fat pads to OA pain. Results from Aikawa et al. identified CGRP-expressing cells in the IPFP from OA patients, which correlated with the severity of OA knee on plain film X-rays as scored with the Kellgren-Lawrence classification. As CGRP expression was stimulated by TNF-α, IL-1β, and PGE2 in the synovial membrane, the study also implied the correlation between CGRP expression and COX2 expression in the IPFP [57]. Another study investigated IPFPs from OA patients who had undergone knee replacement surgery. They found that increased VEGF, MCP-1, and IL-6 protein levels were associated with increased IPFP vascularity in OA samples when compared to those in healthy controls [58].

Preclinical data also supports a role for the IPFP in pain associated with OA. For example, in the rat MIA model of OA, a high concentration of MIA produced persistent inflammation of the fat pad, which was similar to human OA structural changes [59].

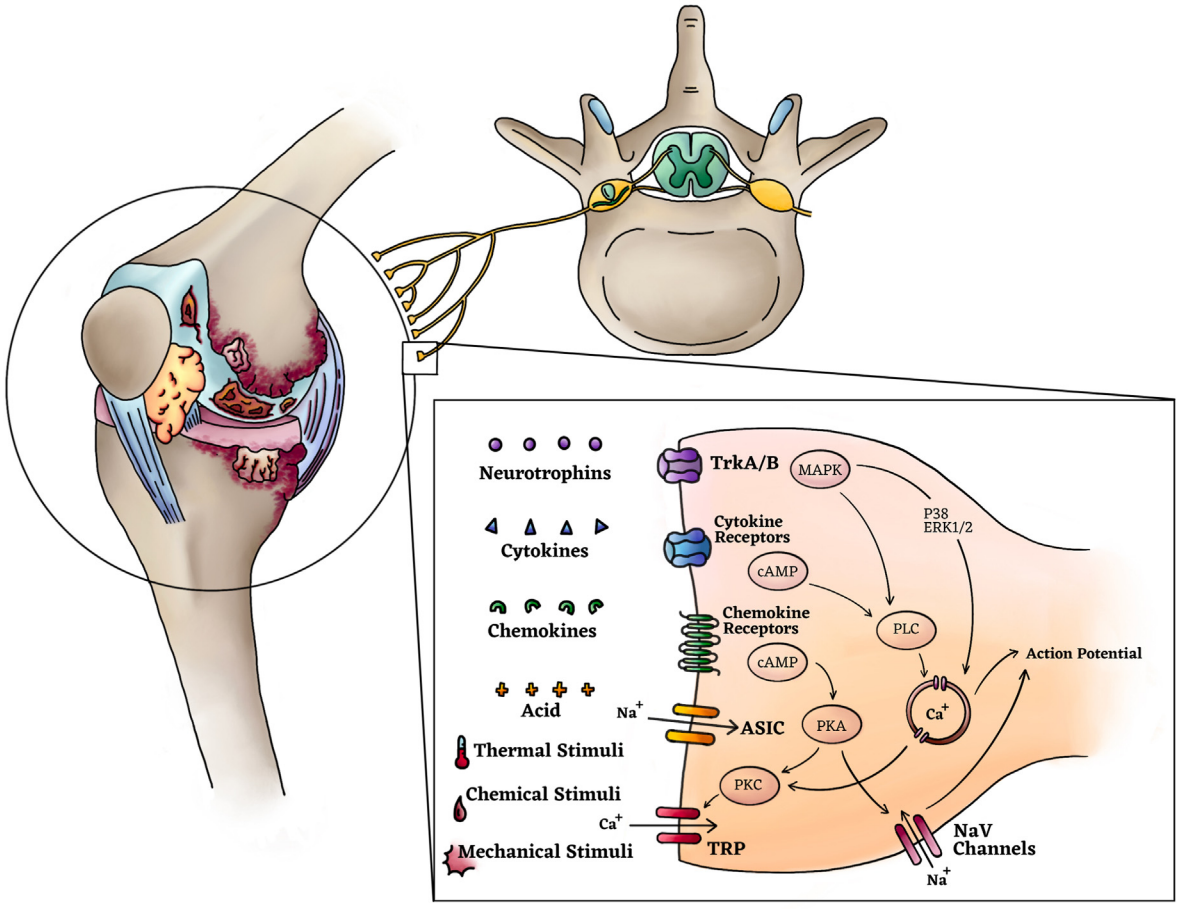


Fig. 1. The pathological changes of OA knee joint and the mechanisms underlying the activation and sensitization of nociceptive afferents. (Acronyms: TrKA-Tropomyosin Receptor Kinase A, ASIC- Acid Sensing Ion Channels, TRP- Transient Receptor Potential Channels, NaV Channels- Sodium Voltage-Gated Channels, MAPK- mitogen-activated protein kinases, cAMP- Cyclic-3′,5′-adenosine monophosphate, PKA- Protein Kinase A, PKC- protein kinase C, PLC- phosphoinositide-specific phospholipase C, ERK- extracellular-signal-regulated kinases, Na- Sodium Ions, Ca- Calcium ions).

3.4. Subchondral bone

The subchondral bone consists of the subchondral bone plate and subchondral trabeculae. The subchondral bone plate lies beneath calcified cartilage providing not only mechanical support but also allowing for molecular interconnections [60]. Bone remodeling through osteoblasts and osteoclasts provides the ability to endure repetitive loading [61]. Subchondral bone alterations, such as the progressive increase in subchondral plate thickness and development of subchondral bone cysts, and formation of osteophytes classically serve as radiographic evidence of OA [62].

In the ACLT mice OA model, Zhu et al. demonstrated that levels of tartrate-resistant acid phosphatase-positive (TRAP-positive) osteoclasts increased in the subchondral bone as a pathological feature of OA correlated with CGRP positive sensory innervation. However, only a few TRAP-positive cells and CGRP-positive sensory neurons were found in the sham group [63]. In addition, this study reported that netrin-1 secreted from osteoclasts was the primary mediator inducing sensory nerve axonal growth; sensory innervation of subchondral bone in the OA model was inhibited by either knock-out of receptor activator of nuclear factor kappa-B ligand mice, knock-out of *Netrin1* gene in TRAP-positive osteoclasts, or alendronate, a drug that inhibited osteoclast activity [63].

In OA patients, osteoclast activity is also associated with the formation of channels that connect subchondral bone to avascular noncalcified cartilage via the tidemark. These channels appear to provide a pathway for the emergence of fibrovascular and infiltrating inflammatory cells, especially macrophages [64]. As with other joint tissues, vascular invasion is associated with sensory innervation. Consistent with this model, there is evidence that the fibrovascular tissue expresses VEGF and NGF and that the vascular channels contain CGRP nerve fibers [44]. CGRP-immunoreactive sensory nerve fibers, found in human OA osteochondral tissues, were increased in osteochondral channels and subchondral bone marrow spaces underneath the damaged cartilage. Moreover, the percentage of nerve fibers was significantly higher in symptomatic than asymptomatic cases [65]. Evidence that these changes in the bone contribute to OA pain comes from a study with resolvin, a pro-resolution lipid mediator [66]. In OA patients, resolvin receptors, ALX and ChemR23, were found in the medial tibial plateau, and receptor levels were significantly correlated with IL-6 levels in the specimens. Notably, while preclinical evidence shows that exogenous systemic and intrathecal administration of resolvin precursors could reduce pain behavior in the MIA OA model, there was no reduction in joint pathology, revealed by unchanged chondropathy grading scores, synovitis levels, and the numbers of TRAP-positive osteoclasts [66].

3.5. Meniscus

Menisci are crescent-shaped wedges of fibrocartilage attached to the tibia beneath the femur in the knee joint. In OA patients, meniscal pathologies such as tearing, maceration, or destruction were often found in MRI imaging [67]. However, Kornick et al. used MRI to examine the meniscal horns in asymptomatic volunteers and found that there was at

least a 25% prevalence of meniscal signal abnormalities as early as the 2 nd decade of life [68], suggesting that meniscal pathology is not directly related to the generation of knee pain. Currently, studies addressing the contribution of menisci to pain are limited. In one study, meniscal structures in high-grade chondropathy showed increased perivascular CGRP-IR nerves in the outer region when compared to low-grade chondropathy. Unfortunately, there were no pain data available [69]. In another study conducted in the mouse DMM OA model, it was reported that nerve fiber immunoreactivity was undetectable in lateral and medial menisci of 10-week-old naive mice but was detectable in the medial meniscus at 16 weeks after DMM [54].

3.6. Ligament

Ligaments are the structures connecting two bones or fibrocartilaginous structures to the bone. The main intraarticular ligaments are the anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL), connecting the femur to the tibia. As noted previously, ACL degeneration and ACL injury are known for their association with OA in humans [70]. Interestingly, in contrast to other joint elements, there is evidence of a decrease in the innervation of the ACL in OA patients. This was suggested to be the cause of chronic dysfunction and the emergence of OA in these patients [71]. However, the contribution of ACL damage to pain in OA patients was not studied. Ikeuchi et al. examined innervation of the PCL in OA and non-OA donors with immunohistochemistry and found that the difference of the nociceptive neuron density, identified by CGRP-positive staining, did not reach a statistical significance between these two groups. However, they revealed that PGP9.5-immunoreactive signals, a general nerve marker, in the PCLs from OA knees were statistically less innervated than non-OA knees [72]. Of note, the relationship between the decrease in total innervations in the PCL and OA pathophysiology has not been demonstrated. Taken together, while these results suggest that a decrease in ligament innervation may contribute to joint instability and, consequently, damage, this is one of the few joint tissues that does not appear to contribute to the pain associated with OA directly.

4. Preclinical models for studying OA pain

Currently, both clinical and experimental studies are conducted to investigate OA pain. Assessing pain in clinical studies usually involves discussing pain intensity history and pain characteristics, physical examinations that focus on pain sensitization such as mechanical temporal summation, pressure pain threshold, and investigations like biomarkers and imaging. These clinical aspects of OA pain have been recently overviewed by Rice et al. [73]. In the present review, we focus on the currently established preclinical models.

Mice and rats are commonly used to study OA pain. Various methods have been developed to create changes in the knee joint that resemble those observed in OA patients [74]. Intra-articular injections of chemicals, such as MIA and collagenase, are associated with changes in the joint and hypersensitivity within a week of injection. The behavioral phenotypes are robust and reproducible, but their clinical relevance is controversial because of the nature of the damage. Consequently, the inflammatory response initiated may not model those observed in OA patients. While more difficult and time-consuming, surgical manipulations have rapidly emerged as the gold standard, at least for models of post-traumatic OA. For example, destabilization of the medial meniscus (DMM) results in joint damage that develops slowly (8–12 weeks). Furthermore, it is associated with minor cartilage damage than medial meniscal transection (MNX) and anterior cruciate ligament transection (ACLT), which are associated with greater joint instability and consequently a more rapid (2 weeks) development of more severe joint damage. As an alternative approach to the generation of more pathophysiologically relevant OA models, investigators have used hyper-physiological joint loading to cause cartilage lesions, which can generate signs and symptoms of OA without a surgical procedure [75]. Lastly, researchers have developed models of spontaneous OA via the manipulation of genetic and natural occurrences. The genetically modified models are based on genes associated with OA pathogenesis, such as protease and collagen type IX α1 genes. A naturally occurring OA model is also believed to closely simulate the natural progression of human primary osteoarthritis [76]. However, these models can take a long time to develop and may induce highly variable disease phenotypes. The detailed description and comparisons of different OA models can be found in a recent article by Alves-Simões [74].

In Fig. 2, we summarize representative animal models used for OA pain research and highlight the methods used to assess pain in rodents. These models have also been used to assess the influence of OA on joint innervation. Pain assessment in small animal models evaluates behavioral responses, facial responses, vocal responses, and spontaneous exploratory activity [77]. Mechanical sensitivity is often assessed with the von Frey test [78], which involves applying a series of calibrated von Frey filament to the plantar surface of animal's foot, and the assessment of the intensity sufficient to make the animal withdraw its foot from the stimulus, or the frequency of withdrawal to repeated applications of the same intensity stimulus. The response to stimuli that would not usually evoke a response implies the presence of mechanical allodynia. Other tests of mechanical sensitivity involve devices such as calipers that can generate pressure on the hind paw or knee. As with the von Frey test, the nociceptive threshold is defined as the intensity of the stimulus needed to evoke a withdrawal and/or vocalization. Decreases in pressure thresholds are consistent with the emergence of hyperalgesia [79]. Indirect measures of pain or sensitivity associated with the joint involve techniques or devices that enable an assessment of ambulation or weight-bearing. The catwalk method or quantitative gait analysis evaluates quantitative parameters of individual and inter-limb coordination [80]. In contrast, ongoing pain is associated with monitoring spontaneous behaviors, such as hindlimb-directed grooming. More recently, investigators have employed the conditioned place preference assay to assess the presence of spontaneous pain, where pain relief, such as that associated with a temporary nerve block, is paired with one side of a two-sided chamber [81]. In contrast, the vehicle is paired with the other. This pairing is done over several days, and on test day, the animals are given free access to both chambers. If animals spend more time on the side paired with pain relief, this change is interpreted to mean that the animals are in spontaneous pain.

Other larger OA animal models (e.g. rabbits, dogs, pigs, sheep, and horses) are also used, which may reflect more complex pathophysiology in humans. Data generated with these models has been well summarized elsewhere [82]. Of note, using these models is more time-consuming and costly than rodents. Therefore, these models have been often used in therapeutic validation studies before clinical trials.

5. Potential of emerging microphysiological system in studying OA pain

As summarized above, animal models are currently the primary tool used to identify mechanisms contributing to OA pain. There are limitations associated with using these models, not the least of which include the difficulty in assessing the presence of pain in a rodent. Furthermore, load distribution and biomechanics in the human knee joint are different in rodents [83]. Given the limited progress in the development of novel therapeutic approaches for treating OA pain, it is reasonable to begin to question the utility of these current models and pain-assessing methods. In particular, considerable effort should be directed at the standardization of OA models, pain outcome measurements, and methodology to facilitate comparisons between different settings.

As an alternative to animal models, investigators have begun to consider the utility of cell/organ culture systems to investigate the mechanisms of OA pain. While isolated neurons alone may suggest potential novel therapeutic targets, these systems fail to account for the fact

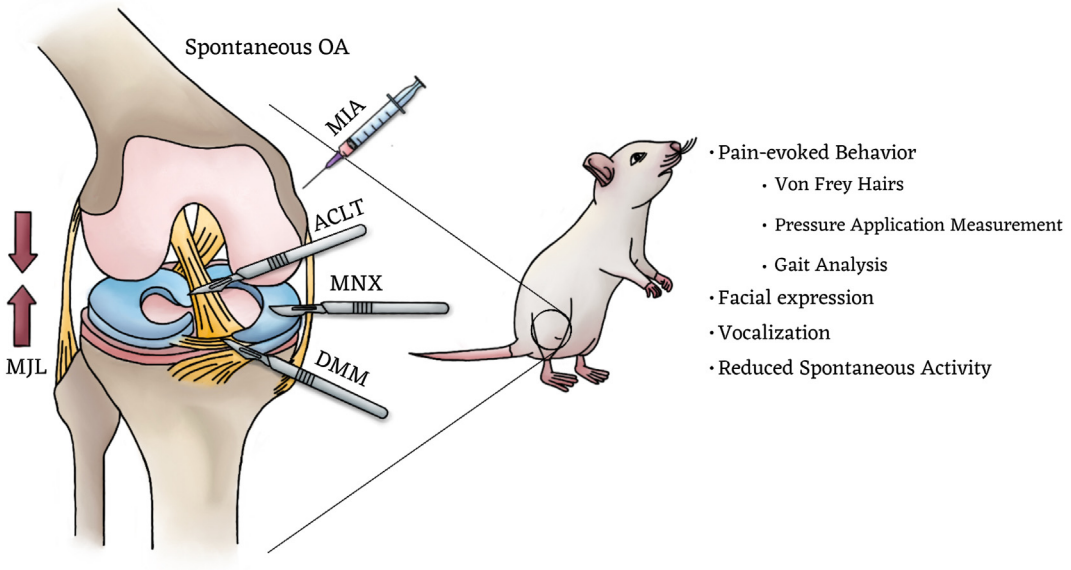


Fig. 2. Current rodent models for studying OA pain. The representative methods to assess pain behavior are also summarized. (Acronyms: MIA-monoiodoacetate injection, ACLT-anterior cruciate ligament transection, MNX-medial meniscal transection, DMM-destabilization of medial meniscus, MJL-mechanical joint loading).

that neurites are embedded within joint tissues and surrounded by cells/matrices that may influence the neural response properties, including both bi-directional influences of joint tissue on the phenotype of neurons and neurons on the phenotype of joint tissue. Therefore, the influence of tissues on neural activity is hard to capture in isolated cell culture models. The limitations of current animal and *in vitro* models suggest that more effective and predictive models of OA are needed. To address these issues, attention has recently been focused on developing clinically relevant microphysiological systems (MPS) or tissue chips [84].

The general concept of MPS is to use human cells to generate miniature and functional organ analogs that simulate the defined human physiology and tissue interactions. Although the use of MPS to study OA pain has not been reported, different types of MPSs were developed to study the interactions of neurons with other cell types. In Table 2, we summarized the studies that included neurons in MPS to inform how to use this emerging system to address OA pain. For example, Neto et al.

Table 2
Studies that used the MPS technology to study neurons and evaluate innervation/pain.

Application	Device name	Cell sources	Culture method	Experiment	References
Axonal degeneration		Mouse DRG neurons	Xona microfluidics	Investigate the function of peripheral axons of DRG	[89]
Bone remodeling		Rat DRG sensory neurons, MC3T3-E1 pre-osteoblastic cell line	PDMS with somal and axonal compartments	Evaluate bone and peripheral axonal regeneration	[85]
Bone remodeling		Rat DRG sensory neurons, rat mesenchymal stem cells	A microfluidic device	Evaluate DRG neurons outgrowth and osteoblastogenesis effect on MSCs	[88]
Bone repair		Rat DRG sensory neuron, Bone marrow-derived endothelial cells	PDMS microfluidic devices	Evaluate sensory neuron effects on endothelial cells angiogenesis and ECM remodeling	[87]
Heart disease	3D3C chips	Human induce Pluripotent stem cells	PDMS microfluidic devices	Generate electrophysiologically functional, human-based cardiac innervation model	[90]
Heart disease	Cardiac sympathetic nervous system	Rat cardiomyocytes and superior cervical ganglions	Organ-chip devices with GelMa hydrogels	Sympathetic innervation increased beat rates of cardiac cells in microfluidic system 3D co-culture	[91]
Inflammatory bone disease	Neuro-vascularized bone chip	Murine embryonic DRG cells, mouse bone marrow cells, Human umbilical vein endothelial cells	PDMS with Fibrin and Collagen/fibroblast hydrogel microchannels	Generate *in vitro* bone microenvironment model, and test drugs (e.g. ibuprofen)	[86]
Motor neuron and muscle degenerative disease		Mice spinal cord explants and myocytes	PDMS microfluidic devices	Investigate neuromuscular junction interaction and activity	[92]
Motor neuron disease		Human embryonic stem-derived motor neuron spheroids and endothelial cells	PDMS with hydrogel channel	Simulate 3D vascular and Motor neuron networks and study the effect of vascular network on synaptic connectivity	[93]
Neuromuscular disorder	hNMJ-on-chip	Human induce pluripotent stem cells and myoblasts from biopsies	PDMS microfluidic chips	Generate a model to study neuronal activity and muscular responses	[94]
Peripheral nerve injury		Mouse C2C12 myoblasts and mice spinal cord (ventral horns) explants	PDMS microfluidic devices	Investigate the effect of promoting contractibility of skeletal muscles on axonal regeneration	[95]
Tooth pain		Embryonic mice trigeminal ganglia and molar tooth germs	A microfluidic device	Investigate the role of innervation in developing teeth	[96]

(Acronyms: MPS- Microphysiological system, DRG- Dorsal root ganglion, PDMS- Polydimethylsiloxane, MSC- Mesenchymal stem cells, ECM- Extracellular matrix, GelMa- Gelatin Methacrylate, 3D3C chips- 3-dimension-three-compartment chips, hNMJ- Human neuromuscular junction).

created a rat DRG-derived sensory neuron-preosteoblastic cell line MC3T3-E1 co-culture model and showed functional sensory nerve growing toward osteoblasts [85]. Similarly, a neurovascularized bone MPS was developed to mimic the bone microenvironment under inflammatory conditions, such as OA and RA. Interestingly, anti-inflammatory drug-loaded nanoparticles were shown to reduce neural growth in this model implying one of the underlying mechanisms of analgesia was to reduce the innervation [86]. Leroux et al. investigated the effects of neuronal networks on angiogenesis and bone extracellular matrix to study bone remodeling. They discovered that sensory neurons, particularly with CGRP and SP neuropeptides treatment, promoted angiogenesis through upregulating the expression of relevant markers, including vascular endothelial growth factor, angiopoietin 1, type IV collagen, and matrix metalloproteinase 2 [87]. Moreover, a microfluidic-based MPS was used to demonstrate a positive effect of neuronal outgrowth on the mesenchymal stem cell osteoblastogenesis [88].

Muscle-nerve chips have been developed to study axon regeneration. In this system, an isolated axonal compartment can be used to identify factors that facilitate regeneration, while an isolated muscle compartment can be used to assess the impact of interventions such as muscle contraction on the regeneration [89,95]. Similarly, MPS with microfluidics has been developed to model neuromuscular disorders, affecting motor neurons (e.g., amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA)), muscles (e.g., Duchenne muscular dystrophy (DMD)), or Schwann cells (e.g., Charcot–Marie–Tooth disease and Guillain–Barré syndrome). All of these systems take advantage of the ability to assess the influence of cell-cell interactions under "naïve" and pathological conditions [92–94]. Other examples of microfluidic-based nerve-tissue MPSs include teeth, where nerve-tooth interactions were studied [96], as well as the heart, where it is possible to study the impact of autonomic nerve dysfunction on heart problems, such as those associated with myocardial infarction and arrhythmia [90,91].

In Fig. 3, based on our recent work in creating a four-tissue miniature joint system [97], we propose an innervated MPS that can be used in studying OA pain. In this model, joint elements that may contribute to pain are included, as well as sensory neurons to innervate the different tissues. Through advanced technologies, such as embedded microelectrode arrays (MEAs), we can monitor the generation of what would be pain signals of minutes, hours, or days, and use this output to test treatments. Moreover, patient cell-derived iPSCs can be used to generate tissue chips, which will allow the development of personalized treatment methods. Nevertheless, while tremendous advances have been made in MPS technology, cell sources, platform design, modeling of human physiology, and simulation of pathological changes in OA still need to be optimized in the future.

In summary, as is true of all scientific endeavors, there are advantages and disadvantages to every model system used. Therefore, the selection of models depends on the questions to be addressed. For example, *in vitro* neural culture can be used to study the direct influences of agents on nerves, while animal models are ideal for assessing the systemic influence of a disease driver or treatments. In order to answer how one tissue, such as synovium, changes nociceptor activity, an innervated MPS could be an ideal platform. In addition, the use of multiple models to validate a novel therapy or targets would allow for a better understanding of the mechanism of treatments and capture the unexpected side effect that cannot be identified in one model. A good previous example is a monoclonal antibody against NGF, which showed promising results in animal studies but unexpectedly accelerated cartilage degradation in human studies. In the future, the potential therapeutics need to be validated in several models from different aspects before they can be moved forward to clinical trials.

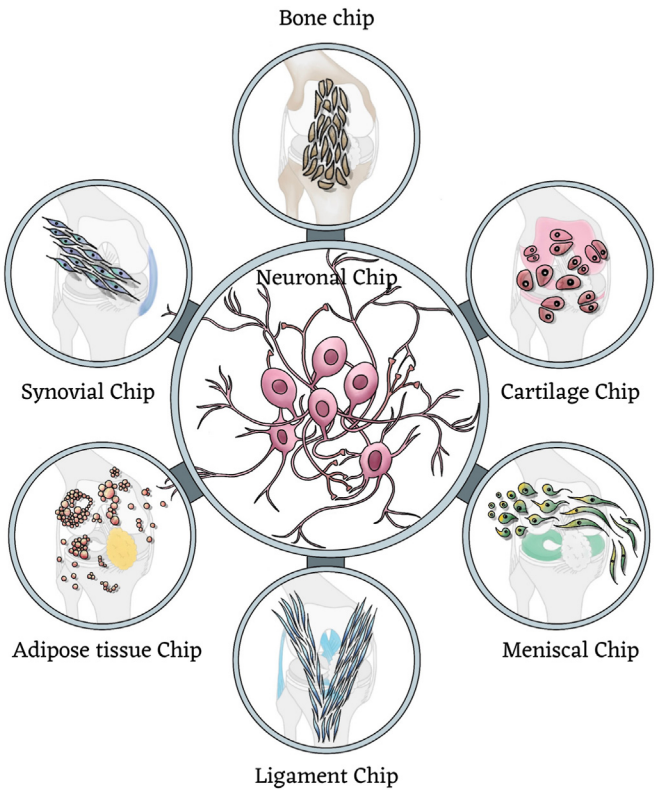


Fig. 3. Tissue chip as a new model for studying OA pain. Here, we propose to create innervated tissue chip to investigate the mechanism of OA pain and development treatments. The joint elements, such as bone, cartilage, meniscus, tendon/ligament, fat, synovium, will be separately created from tissue-specific cells or stem cells, such as mesenchymal stem cells or induced pluripotent stem cells (iPSCs). Moreover, these tissues will be connected through microfluidics to enable crosstalk. Then the neurons along with supporting cells will be seeded into the central chamber and then innervate tissues (except cartilage) through the microchannels in between. Once the pain-enabled tissue chip is established, different insults, such as pro-inflammatory cytokines or mechanical overloading, will be used to induce tissue changes and generate pain-like responses in neurons. In particular, their roles in pain generation can be defined by stimulating different tissues adversely. Collection of medium from different tissues can also lead to finding pain mediators or biomarkers. The system can be used for drug screening as well.

Role of funding sources

This work was supported by the National Institutes of Health (UH3TR002136 to H.L., UG3/UH3TR003090 to M.S.G and H.L.) and the Department of Defense (W81XWH2010902 to H.L.). Dr. Kanyakorn Riewruja is a Prince Mahidol Award Youth Program Scholar, Prince Mahidol Award Foundation under the Royal Patronage. The funding source was not involved in the writing or submission of the manuscript for publication.

Author contribution

KR, MSG, and HL generated the first draft. All authors edited and approved the final manuscript.

Declaration of competing interest

The authors declared no conflicts of interest.

Acknowledgements

The authors thank the funding support from the National Institutes of Health (UH3TR002136 to H.L., UG3/UH3TR003090 to M.S.G. and H.L.), the Department of Defense (W81XWH2010902 to H.L.), and Prince Mahidol Award Foundation under the Royal Patronage.

References

[1] A. Mahmoudian, L.S. Lohmander, A. Mobasheri, M. Englund, F.P. Luyten, Early-stage symptomatic osteoarthritis of the knee - time for action, Nat. Rev. Rheumatol. 17 (2021) 621–632.

[2] T.W. O'Neill, P.S. McCabe, J. McBeth, Update on the epidemiology, risk factors and disease outcomes of osteoarthritis, Best Pract. Res. Clin. Rheumatol. 32 (2018) 312–326.

[3] C. Marc, M. Hochberg, G. Miriam, M. Cisternas, I. Sylvia, P. Watkins-Castillo, He Burden of Musculoskeletal Diseases in the United States (BMUS), fourth ed., 2022.

[4] M.A. Guler, O.F. Celik, F.F. Ayhan, The important role of central sensitization in chronic musculoskeletal pain seen in different rheumatic diseases, Clin. Rheumatol. 39 (2020) 269–274.

[5] R.H. Brophy, Y.A. Fillingham, AAOS Clinical Practice Guideline Summary: Management of Osteoarthritis of the Knee (Nonarthroplasty), third ed., JAAOS, 2022, p. 30.

[6] S.H. Lee, D.H. Kim, Y.S. Lee, Is there an optimal age for total knee arthroplasty?: a systematic review, Knee Surg Relat Res 32 (2020) 60.

[7] W.L. Healy, C.J. Della Valle, R. Iorio, K.R. Berend, F.D. Cushner, D.F. Dalury, et al., Complications of total knee arthroplasty: standardized list and definitions of the Knee Society, Clin. Orthop. Relat. Res. 471 (2013) 215–220.

[8] V. Wylde, A. Beswick, J. Bruce, A. Blom, N. Howells, R. Gooberman-Hill, Chronic pain after total knee arthroplasty, EFORT Open Rev 3 (2018) 461–470.

[9] E. Lluch, R. Torres, J. Nijs, J. Van Oosterwijck, Evidence for central sensitization in patients with osteoarthritis pain: a systematic literature review, Eur. J. Pain 18 (2014) 1367–1375.

[10] A.M. Malfait, R.E. Miller, R.J. Miller, Basic mechanisms of pain in osteoarthritis: experimental observations and new perspectives, Rheum. Dis. Clin. N. Am. 47 (2021) 165–180.

[11] S. Suri, S.E. Gill, S. Massena de Camin, D. Wilson, D.F. McWilliams, D.A. Walsh, Neurovascular invasion at the osteochondral junction and in osteophytes in osteoarthritis, Ann. Rheum. Dis. 66 (2007) 1423–1428.

[12] S.N. Lawson, Phenotype and function of somatic primary afferent nociceptive neurones with C-, Aδ- or Aα/β-fibres, Exp. Physiol. 87 (2002) 239–244.

[13] H.G. Schaible, F. Richter, A. Ebersberger, M.K. Boettger, H. Vanegas, G. Natura, et al., Joint pain, Exp. Brain Res. 196 (2009) 153–162.

[14] J. Kupari, D. Usoskin, M. Parisien, D. Lou, Y. Hu, M. Fatt, et al., Single cell transcriptomics of primate sensory neurons identifies cell types associated with chronic pain, Nat. Commun. 12 (2021) 1510.

[15] R.E. Miller, P.B. Tran, A.M. Obeidat, P. Raghu, S. Ishihara, R.J. Miller, et al., The role of peripheral nociceptive neurons in the pathophysiology of osteoarthritis pain, Curr. Osteoporos. Rep. 13 (2015) 318–326.

[16] Q.Q. Wan, W.P. Qin, Y.X. Ma, M.J. Shen, J. Li, Z.B. Zhang, et al., Crosstalk between bone and nerves within bone, Adv. Sci. 8 (2021), 2003390.

[17] Y.-J. Shao, X. Chen, Z. Chen, H.-Y. Jiang, D.-Y. Zhong, Y.-F. Wang, et al., Sensory nerves protect from the progression of early stage osteoarthritis in mice, Connect. Tissue Res. 61 (2020) 445–455.

[18] A. Courties, J. Sellam, F. Berenbaum, Role of the autonomic nervous system in osteoarthritis, Best Pract. Res. Clin. Rheumatol. 31 (2017) 661–675.

[19] J.J. McDougall, Osteoarthritis is a neurological disease – an hypothesis, Osteoarthritis Cartilage Open 1 (2019), 100005.

[20] S. Grässel, The role of peripheral nerve fibers and their neurotransmitters in cartilage and bone physiology and pathophysiology, Arthritis Res. Ther. 16 (2014) 485.

[21] J.R. Ghilardi, K.T. Freeman, J.M. Jimenez-Andrade, K.A. Coughlin, M.J. Kaczmarska, G. Castaneda-Corral, et al., Neuroplasticity of sensory and sympathetic nerve fibers in a mouse model of a painful arthritic joint, Arthritis Rheum. 64 (2012) 2223–2232.

[22] S. Wright, Treatment of Chronic Pain in the Arthritic with Rehabilitation and Behavioral Medicine Techniques, 1982, p. S148. S148.

[23] A.G. Nackley, K.S. Tan, K. Fecho, P. Flood, L. Diatchenko, W. Maixner, Catechol-O-methyltransferase Inhibition Increases Pain Sensitivity through Activation of Both β2- and β3-adrenergic Receptors, Pain, 2007, p. 128.

[24] A.M. Valdes, A. Abhishek, K. Muir, W. Zhang, R.A. Maciewicz, M. Doherty, Association of beta-blocker use with less prevalent joint pain and lower opioid requirement in people with osteoarthritis, Arthritis Care Res. 69 (2017) 1076–1081.

[25] A. Kessler, M. Yoo, R. Calisoff, Complex regional pain syndrome: an updated comprehensive review, NeuroRehabilitation 47 (2020) 253–264.

[26] M. Fayet, M. Hagen, Pain characteristics and biomarkers in treatment approaches for osteoarthritis pain, Pain Manag. 11 (2021) 59–73.

[27] L. Longobardi, J.D. Temple, L. Tagliafierro, H. Willcockson, A. Esposito, N. D'Onofrio, et al., Role of the C-C chemokine receptor-2 in a murine model of injury-induced osteoarthritis, Osteoarthritis Cartilage 25 (2017) 914–925.

[28] J. Miotla Zarebska, A. Chanalaris, C. Driscoll, A. Burleigh, R.E. Miller, A.M. Malfait, et al., CCL2 and CCR2 regulate pain-related behaviour and early gene expression in post-traumatic murine osteoarthritis but contribute little to chondropathy, Osteoarthritis Cartilage 25 (2017) 406–412.

[29] D.R. Sagar, L. Nwosu, D.A. Walsh, V. Chapman, Dissecting the contribution of knee joint NGF to spinal nociceptive sensitization in a model of OA pain in the rat, Osteoarthritis Cartilage 23 (2015) 906–913.

[30] P.R.W. Gowler, L. Li, S.G. Woodhams, A.J. Bennett, R. Suzuki, D.A. Walsh, et al., Peripheral brain-derived neurotrophic factor contributes to chronic osteoarthritis joint pain, Pain 161 (2020) 61–73.

[31] K.A. Sluka, L.A. Rasmussen, M.M. Edgar, J.M. O'Donnell, R.Y. Walder, S.J. Kolker, et al., Acid-sensing ion channel 3 deficiency increases inflammation but decreases pain behavior in murine arthritis, Arthritis Rheum. 65 (2013) 1194–1202.

[32] K.L. Chu, P. Chandran, S.K. Joshi, M.F. Jarvis, P.R. Kym, S. McGaraughty, TRPV1-related modulation of spinal neuronal activity and behavior in a rat model of osteoarthritic pain, Brain Res. 1369 (2011) 158–166.

[33] Z. Lv, X. Xu, Z. Sun, Y.X. Yang, H. Guo, J. Li, et al., TRPV1 alleviates osteoarthritis by inhibiting M1 macrophage polarization via Ca2+/CaMKII/Nrf2 signaling pathway, Cell Death Dis. 12 (2021) 504.

[34] L.J. Moilanen, M. Hämäläinen, E. Nummenmaa, P. Ilmarinen, K. Vuolteenaho, R.M. Nieminen, et al., Monosodium iodoacetate-induced inflammation and joint pain are reduced in TRPA1 deficient mice–potential role of TRPA1 in osteoarthritis, Osteoarthritis Cartilage 23 (2015) 2017–2026.

[35] S. McGaraughty, K.L. Chu, R.J. Perner, S. Didomenico, M.E. Kort, P.R. Kym, TRPA1 modulation of spontaneous and mechanically evoked firing of spinal neurons in uninjured, osteoarthritic, and inflamed rats, Mol. Pain 6 (2010) 14.

[36] S. Chakrabarti, D.R. Jadon, D.C. Bulmer, E.S.J. Smith, Human osteoarthritic synovial fluid increases excitability of mouse dorsal root ganglion sensory neurons: an in-vitro translational model to study arthritic pain, Rheumatology 59 (2020) 662–667.

[37] M. Soga, T. Izumi, I. Nanchi, N. Horita, M. Yamamoto, S. Kawasaki, et al., Suppression of joint pain in transient receptor potential vanilloid 4 knockout rats with monoiodoacetate-induced osteoarthritis, Pain Rep 6 (2021) e951.

[38] M. Hinata, S. Imai, T. Sanaki, J. Tsuchida, T. Yoshioka, K. Higashino, et al., Sensitization of transient receptor potential vanilloid 4 and increasing its endogenous ligand 5,6-epoxyeicosatrienoic acid in rats with monoiodoacetate-induced osteoarthritis, Pain 159 (2018) 939–947.

[39] W. Rahman, A.H. Dickenson, Osteoarthritis-dependent changes in antinociceptive action of Nav1.7 and Nav1.8 sodium channel blockers: an in vivo electrophysiological study in the rat, Neuroscience 295 (2015) 103–116.

[40] K.P.H. Pritzker, S. Gay, S.A. Jimenez, K. Ostergaard, J.P. Pelletier, P.A. Revell, et al., Osteoarthritis cartilage histopathology: grading and staging, Osteoarthritis Cartilage 14 (2006) 13–29.

[41] M.H.M. Yunus, A. Nordin, H. Kamal, Pathophysiological Perspective of Osteoarthritis vol. 56, Medicina, Kaunas, 2020.

[42] P.R. Coryell, B.O. Diekman, R.F. Loeser, Mechanisms and therapeutic implications of cellular senescence in osteoarthritis, Nat. Rev. Rheumatol. 17 (2021) 47–57.

[43] R. Sohn, G. Rösch, M. Junker, A. Meurer, F. Zaucke, Z. Jenei-Lanzl, Adrenergic signalling in osteoarthritis, Cell. Signal. 82 (2021), 109948.

[44] D.A. Walsh, D.F. McWilliams, M.J. Turley, M.R. Dixon, R.E. Fransès, P.I. Mapp, et al., Angiogenesis and nerve growth factor at the osteochondral junction in rheumatoid arthritis and osteoarthritis, Rheumatology 49 (2010) 1852–1861.

[45] J.M. Teixeira, F. Bobinski, C.A. Parada, K.A. Sluka, C.H. Tambeli, P2X3 and P2X2/3 receptors play a crucial role in articular hyperalgesia development through inflammatory mechanisms in the knee joint experimental synovitis, Mol. Neurobiol. 54 (2017) 6174–6186.

[46] M.D. Smith, The normal synovium, Open Rheumatol. J. 5 (2011) 100–106.

[47] A. Mathiessen, P.G. Conaghan, Synovitis in osteoarthritis: current understanding with therapeutic implications, Arthritis Res. Ther. 19 (2017) 18.

[48] T.A. Nees, N. Rosshirt, J.A. Zhang, H. Platzer, R. Sorbi, E. Tripel, et al., T helper cell infiltration in osteoarthritis-related knee pain and disability, J. Clin. Med. 9 (2020).

[49] I.R. Klein-Wieringa, B.J.E. de Lange-Brokaar, E. Yusuf, S.N. Andersen, J.C. Kwekkeboom, H.M. Kroon, et al., Inflammatory cells in patients with endstage knee osteoarthritis: a comparison between the synovium and the infrapatellar fat pad, J. Rheumatol. 43 (2016) 771–778.

[50] P.L. Zhang, J. Liu, L. Xu, Y. Sun, X.C. Sun, Synovial fluid macrophage migration inhibitory factor levels correlate with severity of self-reported pain in knee osteoarthritis patients, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 22 (2016) 2182–2186.

[51] A. Minatani, K. Uchida, G. Inoue, S. Takano, J. Aikawa, M. Miyagi, et al., Activation of calcitonin gene-related peptide signaling through the prostaglandin E2-EP1/EP2/EP4 receptor pathway in synovium of knee osteoarthritis patients, J. Orthop. Surg. Res. 11 (2016) 117.

[52] T. Hoshino, K. Tsuji, H. Onuma, M. Udo, H. Ueki, M. Akiyama, et al., Persistent synovial inflammation plays important roles in persistent pain development in the rat knee before cartilage degradation reaches the subchondral bone, BMC Muscoskel. Disord. 19 (2018) 291.

[53] V. Bourassa, H. Deamond, N. Yousefpour, M.A. Fitzcharles, A. Ribeiro-da-Silva, Pain-related behavior is associated with increased joint innervation, ipsilateral dorsal horn gliosis, and dorsal root ganglia activating transcription factor 3 expression in a rat ankle joint model of osteoarthritis, Pain Rep 5 (2020) e846.

[54] A.M. Obeidat, R.E. Miller, R.J. Miller, A.M. Malfait, The nociceptive innervation of the normal and osteoarthritic mouse knee, Osteoarthritis Cartilage 27 (2019) 1669–1679.

[55] A. Bratus-Neuenschwander, F. Castro-Giner, M. Frank-Bertoncelj, S. Aluri, S.F. Fucentese, R. Schlapbach, et al., Pain-associated transcriptome changes in synovium of knee osteoarthritis patients, Genes 9 (2018).

[56] M. Favero, H. El-Hadi, E. Belluzzi, M. Granzotto, A. Porzionato, G. Sarasin, et al., Infrapatellar fat pad features in osteoarthritis: a histopathological and molecular study, Rheumatology 56 (2017) 1784–1793.

[57] J. Aikawa, K. Uchida, S. Takano, G. Inoue, D. Iwase, M. Miyagi, et al., Regulation of calcitonin gene-related peptide expression through the COX-2/mPGES-1/PGE2 pathway in the infrapatellar fat pad in knee osteoarthritis, Lipids Health Dis. 17 (2018) 215.

[58] E. Belluzzi, V. Macchi, C.G. Fontanella, E.L. Carniel, E. Olivotto, G. Filardo, et al., Infrapatellar fat pad gene expression and protein production in patients with and without osteoarthritis, Int. J. Mol. Sci. 21 (2020) 6016.

[59] K. Inomata, K. Tsuji, H. Onuma, T. Hoshino, M. Udo, M. Akiyama, et al., Time course analyses of structural changes in the infrapatellar fat pad and synovial

membrane during inflammation-induced persistent pain development in rat knee joint, BMC Muscoskel. Disord. 20 (2019) 8.
[60] X. Zhu, Y.T. Chan, P.S.H. Yung, R.S. Tuan, Y. Jiang, Subchondral bone remodeling: a therapeutic target for osteoarthritis, Front. Cell Dev. Biol. 8 (2020), 607764.
[61] H.L. Stewart, C.E. Kawcak, The importance of subchondral bone in the pathophysiology of osteoarthritis, Front. Vet. Sci. 5 (2018).
[62] D.J. Hunter, A. Guermazi, F. Roemer, Y. Zhang, T. Neogi, Structural correlates of pain in joints with osteoarthritis, Osteoarthritis Cartilage 21 (2013) 1170–1178.
[63] S. Zhu, J. Zhu, G. Zhen, Y. Hu, S. An, Y. Li, et al., Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain, J. Clin. Invest. 129 (2019) 1076–1093.
[64] P.I. Mapp, D.A. Walsh, Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis, Nat. Rev. Rheumatol. 8 (2012) 390–398.
[65] K. Aso, S.M. Shahtaheri, R. Hill, D. Wilson, D.F. McWilliams, L.N. Nwosu, et al., Contribution of nerves within osteochondral channels to osteoarthritis knee pain in humans and rats, Osteoarthritis Cartilage 28 (2020) 1245–1254.
[66] J. Huang, J.J. Burston, L. Li, S. Ashraf, P.I. Mapp, A.J. Bennett, et al., Targeting the D Series resolvin receptor system for the treatment of osteoarthritis pain, Arthritis Rheumatol. 69 (2017) 996–1008.
[67] M. Englund, A. Guermazi, S.L. Lohmander, The role of the meniscus in knee osteoarthritis: a cause or consequence? Radiol. Clin. 47 (2009) 703–712.
[68] J. Kornick, E. Trefelner, S. McCarthy, R. Lange, K. Lynch, P. Jokl, Meniscal abnormalities in the asymptomatic population at MR imaging, Radiology 177 (1990) 463–465.
[69] S. Ashraf, H. Wibberley, P.I. Mapp, R. Hill, D. Wilson, D.A. Walsh, Increased vascular penetration and nerve growth in the meniscus: a potential source of pain in osteoarthritis, Ann. Rheum. Dis. 70 (2011) 523–529.
[70] G. Schulze-Tanzil, Intraarticular ligament degeneration is interrelated with cartilage and bone destruction in osteoarthritis, Cells 8 (2019).
[71] C.V. Nagelli, J.L. Cook, K. Kuroki, C. Bozynski, R. Ma, T.E. Hewett, Does anterior cruciate ligament innervation matter for joint function and development of osteoarthritis? J. Knee Surg. 30 (2017) 364–371.
[72] M. Ikeuchi, Q. Wang, M. Izumi, T. Tani, Nociceptive sensory innervation of the posterior cruciate ligament in osteoarthritic knees, Arch. Orthop. Trauma. Surg. 132 (2012) 891–895.
[73] D. Rice, P. McNair, E. Huysmans, J. Letzen, P. Finan, Best evidence rehabilitation for chronic pain Part 5: osteoarthritis, J. Clin. Med. 8 (2019).
[74] M. Alves-Simoes, Rodent models of knee osteoarthritis for pain research, Osteoarthritis Cartilage 30 (6) (2022) 802–814.
[75] F. Ter Heegde, A.P. Luiz, S. Santana-Varela, I.P. Chessell, F. Welsh, J.N. Wood, et al., Noninvasive mechanical joint loading as an alternative model for osteoarthritic pain, Arthritis Rheumatol. 71 (2019) 1078–1088.
[76] E.L. Kuyinu, G. Narayanan, L.S. Nair, C.T. Laurencin, Animal models of osteoarthritis: classification, update, and measurement of outcomes, J. Orthop. Surg. Res. 11 (2016) 19.
[77] M.J. Piel, J.S. Kroin, A.J. van Wijnen, R. Kc, H.-J. Im, Pain assessment in animal models of osteoarthritis, Gene 537 (2014) 184–188.
[78] B. Tena, B. Escobar, M.J. Arguis, C. Cantero, J. Rios, C. Gomar, Reproducibility of electronic von Frey and von Frey monofilaments testing, Clin. J. Pain 28 (2012) 318–323.
[79] R.V. Kamath, G. Simler, C. Zhou, M. Hart, S. Joshi, P. Honore, Development and validation of mechanical allodynia as a pain readout in a preclinical model of osteoarthritis, Osteoarthritis Cartilage 20 (2012) S62.
[80] J. Ferreira-Gomes, S. Adães, J.M. Castro-Lopes, Assessment of movement-evoked pain in osteoarthritis by the knee-bend and CatWalk tests: a clinically relevant study, J. Pain 9 (2008) 945–954.
[81] J. Havelin, I. Imbert, J. Cormier, J. Allen, F. Porreca, T. King, Central sensitization and neuropathic features of ongoing pain in a rat model IKGof advanced osteoarthritis, J. Pain 17 (2016) 374–382.
[82] I. Ribitsch, P.M. Baptista, A. Lange-Consiglio, L. Melotti, M. Patruno, F. Jenner, et al., Large animal models in regenerative medicine and tissue engineering: to do or not to do, Front. Bioeng. Biotechnol. 8 (2020) 972.
[83] E. Teeple, G.D. Jay, K.A. Elsaid, B.C. Fleming, Animal models of osteoarthritis: challenges of model selection and analysis, AAPS J. 15 (2013) 438–446.
[84] M.J. Makarczyk, Q. Gao, Y. He, Z. Li, M.S. Gold, M.C. Hochberg, et al., Current models for development of disease-modifying osteoarthritis drugs, Tissue Eng. C Methods 27 (2021) 124–138.
[85] E. Neto, C.J. Alves, D.M. Sousa, I.S. Alencastre, A.H. Lourenço, L. Leitão, et al., Sensory neurons and osteoblasts: close partners in a microfluidic platform, Integr Biol (Camb) 6 (2014) 586–595.
[86] E. Neto, A.C. Monteiro, C. Leite Pereira, M. Simões, J.P. Conde, V. Chu, et al., Micropathological chip modeling the neurovascular unit response to inflammatory bone condition, Adv Healthc Mater 11 (2022), e2102305.
[87] A. Leroux, B. Paiva Dos Santos, J. Leng, H. Oliveira, J. Amédée, Sensory neurons from dorsal root ganglia regulate endothelial cell function in extracellular matrix remodelling, Cell Commun. Signal. 18 (2020) 162.
[88] D.I. Silva, B.P.D. Santos, J. Leng, H. Oliveira, J. Amédée, Dorsal root ganglion neurons regulate the transcriptional and translational programs of osteoblast differentiation in a microfluidic platform, Cell Death Dis. 8 (2017) 3209.
[89] R. Atmaramani, S. Veeramachaneni, L.V. Mogas, P. Koppikar, B.J. Black, A. Hammack, et al., Investigating the function of adult DRG neuron axons using an in vitro microfluidic culture system, Micromachines 12 (2021).
[90] M. Häkli, S. Jäntti, T. Joki, L. Sukki, K. Tornberg, K. Aalto-Setälä, et al., Human neurons form axon-mediated functional connections with human cardiomyocytes in compartmentalized microfluidic chip, Int. J. Mol. Sci. 23 (2022).
[91] J.R. Soucy, A.J. Bindas, R. Brady, T. Torregrosa, C.M. Denoncourt, S. Hosic, et al., Reconfigurable microphysiological systems for modeling innervation and multitissue interactions, Adv Biosyst 4 (2020), e2000133.
[92] A. Ionescu, E.E. Zahavi, T. Gradus, K. Ben-Yaakov, E. Perlson, Compartmental microfluidic system for studying muscle-neuron communication and neuromuscular junction maintenance, Eur. J. Cell Biol. 95 (2016) 69–88.
[93] T. Osaki, V. Sivathanu, R.D. Kamm, Engineered 3D vascular and neuronal networks in a microfluidic platform, Sci. Rep. 8 (2018) 5168.
[94] P. Duc, M. Vignes, G. Hugon, A. Sebban, G. Carnac, E. Malyshev, et al., Human neuromuscular junction on micro-structured microfluidic devices implemented with a custom micro electrode array (MEA), Lab Chip 21 (2021) 4223–4236.
[95] J. Sala-Jarque, F. Mesquida-Veny, M. Badiola-Mateos, J. Samitier, A. Hervera, J.A. Del Río, Neuromuscular activity induces paracrine signaling and triggers axonal regrowth after injury in microfluidic lab-on-chip devices, Cells 9 (2020).
[96] P. Pagella, E. Neto, L. Jiménez-Rojo, M. Lamghari, T.A. Mitsiadis, Microfluidics co-culture systems for studying tooth innervation, Front. Physiol. 5 (2014) 326.
[97] Z. Li, Z. Lin, S. Liu, H. Yagi, X. Zhang, L. Yocum, et al., Human mesenchymal stem cell-derived miniature joint system for disease modeling and drug testing, Adv. Sci. (2022), e2105909.