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Inflammation and fibrosis induced by joint remobilization, and relevance to progression of arthrogenic joint contracture: A narrative review

Akinori Kaneguchi[1] and Junya Ozawa[1]*

[1] Department of Rehabilitation, Faculty of Rehabilitation, Hiroshima International University, Kurose-Gakuendai 555-36, Higashi-Hiroshima, Hiroshima, Japan

* Corresponding author
Junya Ozawa
Department of Rehabilitation, Faculty of Rehabilitation, Hiroshima International University, Kurose-Gakuendai 555-36, Higashi-Hiroshima, Hiroshima, 739-2695, Japan
E-mail: j-ozawa@hirokoku-u.ac.jp
Tel: +81-823-70-4547
Fax: +81-823-70-4542

Short title: Remobilization-induced joint inflammation and fibrosis

Summary

Joint immobilization is frequently administered after fractures and ligament injuries and can cause joint contracture as a side effect. The structures responsible for immobilization-induced joint contracture can be roughly divided into muscular and articular. During remobilization, although myogenic contracture recovers spontaneously, arthrogenic contracture is irreversible or deteriorates further. Immediately after remobilization, an inflammatory response is observed, characterized by joint swelling, deposit formation in the joint space, edema, inflammatory cell infiltration, and the upregulation of genes encoding proinflammatory cytokines in the joint capsule. Subsequently, fibrosis in the joint capsule develops, in parallel with progressing arthrogenic contracture. The triggers of remobilization-induced joint inflammation are not fully understood, but two potential mechanisms are proposed: 1) micro-damage induced by mechanical stress in the joint capsule, and 2) nitric oxide (NO) production via NO synthase 2. Some interventions can modulate remobilization-induced inflammatory and subsequent fibrotic reactions. Anti-inflammatory treatments, such as steroidal anti-inflammatory drugs and low-level laser therapy, can attenuate joint capsule fibrosis and the progression of arthrogenic contracture in remobilized joints. Antiproliferative treatment using the cell-proliferation inhibitor mitomycin C can also attenuate joint

capsule fibrosis by inhibiting fibroblast proliferation without suppressing inflammation. Conversely, aggressive exercise during the early remobilization phases is counterproductive, because it facilitates inflammatory and then fibrotic reactions in the joint. However, the adverse effects of aggressive exercise on remobilization-induced inflammation and fibrosis are offset by anti-inflammatory treatment. To prevent the progression of arthrogenic contracture during remobilization, therefore, care should be taken to control inflammatory and fibrotic reactions in the joints.

Key words: Joint immobilization, Joint remobilization, Joint contracture, Inflammation, Fibrosis

Introduction: immobilization-induced joint contracture

Joint immobilization is frequently administered after fractures and ligament injuries to maintain the resting state of injured tissues [1-5]. However, it has the side effect of causing joint contracture, muscle atrophy, articular cartilage degeneration, and reduced bone mineral density [2-6]. Immobilization-induced joint contracture induces pain, the increase in risk of falls, and pressure ulcers, which contribute to long-term sequelae [7]. Prevention and/or improvement of immobilization-induced joint contracture are thus critical issues in rehabilitation medicine. Several studies using animal models have investigated the pathophysiology of immobilization-induced joint contracture. Among these, knee flexion contracture models induced by immobilization in a flexed position are the most common [8-18]. Previous studies using these animal models had revealed that the structures responsible for immobilization-induced joint contracture can be roughly divided into muscular and articular structures [16,17]. Muscular structures are mainly responsible for short-term (less than four weeks) immobilization-induced joint contracture, while articular structures, especially the joint capsule, play a central role in prolonged (four or more weeks) immobilization-induced joint contracture [8,12,15-17]. After the injured tissues have healed, joints are released from immobilization, i.e., they are remobilized [3-5]. During remobilization, myogenic contracture

recovers spontaneously, but arthrogenic contracture is generally irreversible [11,16,19,20]. Surprisingly, arthrogenic contracture deteriorates further during remobilization following immobilization for three weeks or less [16,21-26]. For instance, during remobilization following three weeks of immobilization, range of motion (ROM) before myotomy, which mainly reflects myogenic factors, recovers partially [22]. In contrast, after myotomy, which reflects arthrogenic factors, ROM decreases further [22]. Progression of arthrogenic contracture during remobilization should thus be targeted to avoid irreversible joint contracture. In clinical practice, passive stretching is frequently performed to treat immobilization-induced joint contractures [7]. However, a randomized controlled trial revealed that passive stretching after cast immobilization for ankle fracture does not improve ankle plantar flexion contracture. [2] Thus, developing new therapeutic strategies for immobilization-induced joint contracture is an important issue. An understanding of the natural course of intra-articular changes during remobilization is crucial for developing therapeutic strategies. In this review, we describe the natural course of intraarticular changes during remobilization and its modification by some interventions.

The natural course of intra-articular changes during remobilization

Joint inflammation is observed during the early phases of immobilization (within two weeks), but this inflammation is transient and subsides thereafter [13,27-29]. Accordingly, signs of inflammation were not detected in the rat joint capsule after three weeks of knee immobilization [22]. On day 1 of remobilization, however, an inflammatory response characterized by joint swelling, deposit formation in the joint space, edema, inflammatory cell infiltration, upregulation of genes encoding the proinflammatory cytokines interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) in the joint capsule was detected [22,25,30]. Similarly, Michelsson and Hunneyball reported development of synovitis during remobilization in the rabbit knee after five weeks of immobilization [31]. Following the inflammatory response, increased bromodeoxyuridine (BrdU)-positive cells in the joint capsule, which signify proliferating cells, which peaked on day 3 of remobilization was observed [22]. Fibroblasts isolated from the synovium express high levels of IL-1 receptors [32] and proliferate in response to IL-1β [33]. Therefore, increased BrdU-positive cells may represent fibroblast proliferation in response to IL-1β upregulation. Indeed, a significant increase in fibroblasts on day 7 of remobilization was observed [23,24,26]. Moreover, day 7 of remobilization was characterized by increased expression levels of the gene encoding the profibrotic cytokine transforming growth factor-β1 (TGF-β1) [22]. It is well known that TGF-β1 stimulates the differentiation of fibroblasts

into myofibroblasts and enhances the synthesis of matrix proteins such as collagen from these cells [34]. Accordingly, increased numbers of myofibroblasts and the upregulation of type I (*COL1A1*) and III (*COL3A1*) collagen genes were also detected on day 7 of remobilization [22,25]. Consequently, joint capsule fibrosis characterized by densely packed capsules via overexpression of type I and III collagen and the shortening of synovial lengths was observed [22-26]. Fibrosis of joint components is considered a main cause of arthrogenic contracture [15,35,36], and supporting this, the progression of arthrogenic contracture was observed in parallel with remobilization-induced joint capsule fibrosis [22-26]. These results highlight the importance of preventing remobilization-induced joint inflammation and subsequent fibrosis for blocking arthrogenic contracture progression.

The following two triggers of remobilization-induced joint inflammation are proposed, although the mechanisms are not fully understood: 1) micro-damage induced by mechanical stress in the joint capsule, and 2) nitric oxide (NO) production via NO synthase (NOS) 2. On day 1 of remobilization following three weeks of knee immobilization in a flexed position, extravascular erythrocytes (i.e., internal bleeding) were observed histologically in the posterior joint capsule [22]. This finding suggests the presence of micro-damage in the joint capsule, which may trigger remobilization-induced joint inflammation [22], although

103 the possibility that erythrocytes had leaked out due to increased vascular permeability associated with
104 inflammation cannot be excluded. Damage in the posterior joint capsule has crucial roles for the
105 development of knee flexion contracture. Knee hyperextension followed by immobilization induced
106 posterior joint capsule damage characterized by inflammatory cell infiltration with fibrosis in rats [37]. The
107 effect of posterior joint capsule damage on knee joint contracture induced by immobilization after cortical
108 bone removal were examined in rabbits. As a result, immobilization with capsule damage induced by knee
109 hyperextension caused more severe contracture compared with immobilization only [38]. These findings
110 suggest that joint capsule damage, even if the damage is minor, triggers inflammation and subsequent
111 fibrosis that can aggravate arthrogenic contractures. The knee posterior joint capsule (synovium) is
112 shortened by joint immobilization in a flexed position [22-25,39,40]. In addition, knee immobilization in
113 the flexed position induces the thinning of collagen fiber bundles in the posterior joint capsule, suggesting
114 weakening of the posterior joint capsule [41]. The tensile stress generated by remobilization on the
115 shortened and weakened joint capsule may cause the micro-damage [22]. Therefore, aggressive active
116 exercise or violent passive joint movement for immobilized joints will induce or facilitate joint
117 inflammation via micro-damage in the joint capsule. In fact, a previous study reported that forced

118 remobilization (abrupt movement through the full ROM immediately after removal of the fixator followed
119 by free remobilization) induced a tear intra-articular connective tissue accompanied by bleeding [42]. In
120 addition, other previous study indicated that intermittent violent exercise (exercise using the full ROM)
121 during rabbit knee immobilization was injurious and aggravated joint contracture and swelling [43].

122 The NO synthesized via NOS2 is considered an important mediator of the pathogenesis of
123 inflammatory diseases, such as rheumatoid arthritis and osteoarthritis, in joints [44]. *NOS2* in the joint
124 capsule was upregulated on day 1 of remobilization after three weeks of immobilization [30]. The NOS
125 inhibitor L-NG-nitroarginine methyl ester (L-NAME) administration before and during remobilization can
126 attenuate several aspects of the inflammatory response: joint swelling, inflammatory cell infiltration, edema,
127 and upregulation of *TNF-α* in the joint capsule [30]. These results suggest that NO production via NOS2
128 contributes, at least in part, to remobilization-induced joint inflammation.

129 In osteoarthritic joints, hypoxia/reoxygenation is an underlying mechanism that induces NOS2 [45].
130 Previous studies reported that joint immobilization induces hypoxic conditions in the joint capsule [29,46].
131 However, another study investigated the expression of hypoxia marker gene *hypoxia inducible factor-1α*
132 (*HIF-1α*) in the joint capsule during immobilization and remobilization in rat knee joints and reported that

133 the expression of *HIF-1α* was not upregulated by immobilization, but was instead upregulated by
134 remobilization [30]. Therefore, the mechanisms behind remobilization-induced NOS2 upregulation may
135 not stem from hypoxia/reoxygenation. Further research is needed to identify these mechanisms.
136
137 **Effects of interventions on inflammation and fibrosis**
138 Anti-inflammatory therapies
139 Inflammation can trigger fibrosis in various organs, including joints [47,48]. It is speculated that anti-
140 inflammatory therapies during joint remobilization can prevent fibrosis and the subsequent progression of
141 arthrogenic contracture. A previous study examined the effects of the steroidal anti-inflammatory drug
142 dexamethasone during remobilization on joint capsule fibrosis and arthrogenic contracture progression. The
143 anti-inflammatory effects of subcutaneous injections of dexamethasone were confirmed by a complete
144 blockade of upregulation of *IL-1β* and *IL-6* in the joint capsule and joint swelling on day 1 of remobilization
145 following three weeks of immobilization [25]. Thereafter, dexamethasone prevented increases in
146 myofibroblasts, overexpression of type I and III collagen at both the gene and the protein level, and
147 shortening of the synovium on day 7 of remobilization [25]. Progression of arthrogenic contracture during

remobilization was thus completely prevented by this treatment [25]. These results confirm that inflammation is a trigger for fibrosis in the remobilized joint, which induces arthrogenic contracture progression.

Steroidal anti-inflammatory drugs have strong anti-inflammatory effects, but also many side effects, including muscle atrophy and osteoporosis [49]. The effects of low-level laser therapy (LLLT), which has anti-inflammatory and anti-fibrotic effects with few adverse side effects, on remobilization-induced joint fibrosis and progression of arthrogenic contracture was also tested. Only 120 s/day of LLLT during remobilization attenuated fibrotic reactions in the joint capsule and progression of arthrogenic contracture, although whether remobilization-induced joint inflammation was prevented by LLLT was not confirmed [21]. A previous study reported that LLLT for cultured synoviocytes from rheumatoid arthritis patients decreased expression of IL-1β and TNF-α at both the gene and the protein level [50]. In post-surgical knee joint contracture model, it is confirmed that LLLT could downregulate the gene expression of *IL-1β* in the joint capsule [51]. Thus, LLLT will attenuate fibrosis through inhibition of remobilization-induced joint inflammation. In addition, LLLT for cultured fibroblasts can attenuate the fibrotic reactions in the pro-fibrotic environments. For instance, LLLT on murine embryonic fibroblasts stimulated with TGF-β1

decreased expression of TGF-β and type I collagen proteins [52]. Therefore, LLLT may suppress the fibrotic reactions not only through indirect mechanisms via anti-inflammatory effects, but also through direct mechanisms. Combined, these results indicate that anti-inflammatory therapies during remobilization are effective for preventing joint fibrosis and progression of arthrogenic contracture.

Exercise

Clinically, it is generally believed that aggressive exercise is effective for preventing or improving joint contracture. However, recent reviews suggest that if inflammation is not well controlled, aggressive exercise soon after joint surgery can lead to joint fibrosis and contracture formation by enhancing inflammation [53,54]. Therefore, aggressive exercise during the early phases of remobilization, when joint inflammation occurs, may cause the progression of arthrogenic contracture by enhancing inflammatory and fibrotic reactions in the joints. This possibility was tested by examining the effects of treadmill exercise on remobilized rat knee joints. Treadmill exercise (12 m/min, 60 min/day) performed immediately after remobilization following three weeks of immobilization upregulated the proinflammatory *IL-1β* gene in the joint capsule on day 1 [23]. By day 7 of remobilization, the daily treadmill exercise had caused an increase

178 in fibrotic reactions in the joint capsule, characterized by upregulation of the profibrotic *TGF-β1* gene,
179 fibroblast proliferation, and increased type I and III collagen at both the gene and the protein level, which
180 led to progression of arthrogenic contracture [23]. These results indicate that aggressive exercise during the
181 early phases of remobilization aggravates arthrogenic contracture by enhancing inflammatory and
182 subsequent fibrotic reactions in the joints.

183 However, exercise during joint remobilization is indispensable for recovering muscle mass,
184 muscle strength, and daily activities [3-5]. A previous study investigated whether anti-inflammatory
185 treatments combined with exercise can offset the adverse effects of exercise during the early phases of
186 remobilization on inflammatory and subsequent fibrotic reactions. When anti-inflammatory LLLT was
187 combined with treadmill exercises, the enhancement of inflammatory and subsequent fibrotic reactions by
188 treadmill exercise was attenuated, and arthrogenic contracture during remobilization was completely
189 prevented [24]. These results suggest that if exercise during the early phases of joint remobilization is
190 essential, it should be combined with anti-inflammatory treatments to offset the adverse effects of exercise
191 on inflammatory and subsequent fibrotic reactions in the joints.
192

193 Antiproliferative agent

194 Because fibroblasts produce extracellular matrix proteins, such as collagens, the proliferation of this type
195 of cell is important part of the development of fibrosis in various organs, including joints [55,56]. Therefore,
196 remobilization-induced joint fibrosis may be blocked by the inhibition of fibroblast proliferation,
197 irrespective of whether inflammation is prevented. To test this possibility, a previous study tested the effects
198 of cell proliferation inhibitor mitomycin C (MMC) on fibroblast proliferation as well as joint capsule
199 fibrosis [26]. MMC is used as an anticancer drug in clinical practice [57], but is also used to inhibit
200 fibroblast proliferation in animals and *in vitro* experiments [58-60]. Because cell proliferation peaks three
201 days following joint remobilization [22], intra-articular injections of MMC were performed immediately
202 after and three days after remobilization [26]. As a result, fibroblast proliferation and joint capsule fibrosis
203 during remobilization were partially attenuated, which prevented the progression of arthrogenic contracture
204 [26]. These results indicate that fibroblast proliferation triggered by inflammation mediates joint capsule
205 fibrosis, which induces the progression of arthrogenic contracture in remobilized joints. Therefore, both
206 inflammation and the subsequent fibroblast proliferation are potential therapeutic targets for preventing
207 remobilization-induced joint fibrosis and the resulting progression of arthrogenic contracture.

208

209 **Future directions**

210 The anti-inflammatory and antiproliferative treatments featured here can attenuate joint
211 remobilization-induced inflammation, fibrosis, and arthrogenic contracture progression, but cannot restore
212 joint contracture to normal levels. To prevent permanent joint contracture, future studies should develop
213 more-effective treatment strategies and/or combinations of multiple treatments, including preventive
214 intervention during immobilization. Joint immobilization periods vary among types of injured tissue and
215 the degree of injury. The immobilization periods in previous studies reporting remobilization-induced joint
216 inflammation were three or five weeks [22-25,30,31]. Future studies should examine whether inflammatory
217 and fibrotic reactions are induced by remobilization following shorter- or longer-term joint immobilization.
218 In addition, most of the findings in this review were derived from basic research using young animals.
219 Further studies are needed to confirm whether similar reactions occur in human patients. Accumulation of
220 advanced-glycation end products in the joint is detected in the elderly [61] and amplifies inflammatory
221 changes induced by immobilization [13]. In elderly patients, thus, immobilization-induced joint contracture
222 may be aggravated, and recovery from joint contracture may be difficult compared with young patients.

These possibilities should be tested in future studies.

Conclusion

To prevent permanent joint contracture, treatments for arthrogenic contracture during remobilization are indispensable. After joint remobilization, inflammation, fibrosis, and the subsequent progression of arthrogenic contracture occur within seven days (Fig. 1). Therefore, inflammatory and fibrotic reactions should be controlled by qualified professionals such as physiotherapists, especially during the early stage of remobilization. Anti-inflammatory and antiproliferative treatments are effective for preventing inflammatory and/or fibrotic reactions. Conversely, aggressive exercise during the early phases of remobilization is counterproductive, since it facilitates inflammatory and then fibrotic reactions in the joints. Combining exercises during the early phases of joint remobilization that are essential for recovering muscle mass, strength, and daily activities with anti-inflammatory treatments such as LLLT and anti-inflammatory drugs may limit excess inflammation.

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239

240 **Declaration of Conflicting Interests**

241 The Authors declare that there is no conflict of interest.

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Figure 1

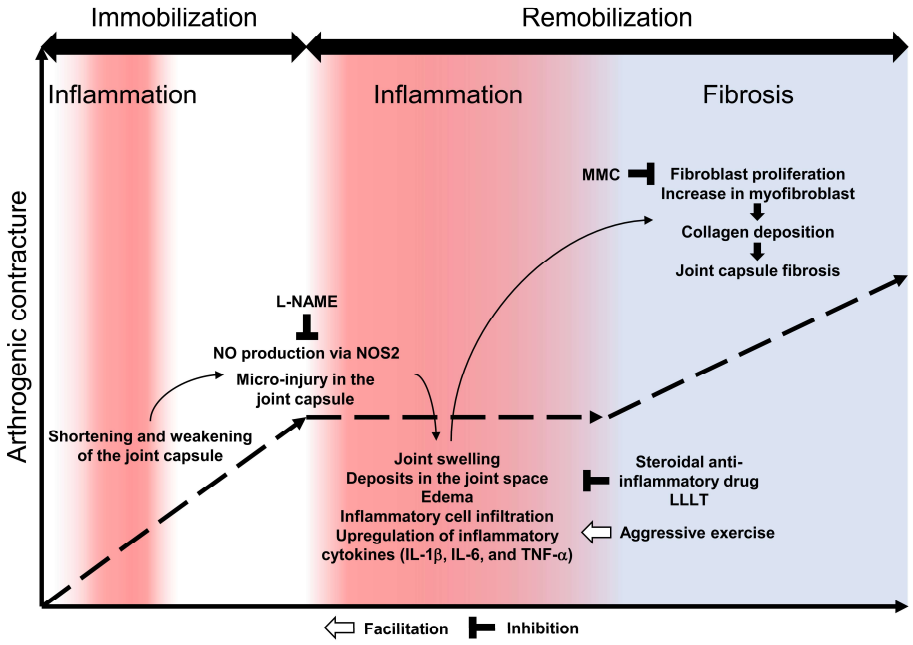

Figure legend

430 **Fig. 1** Schema illustrating intra-articular changes during immobilization and remobilization.

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