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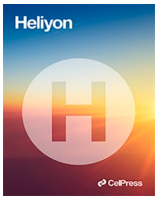

Review article

Rat shoulder contracture models: Techniques, evaluation, pathophysiology, and applications in developing treatment interventions

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ABSTRACT

Studies on the pathophysiology of shoulder contracture and development of interventions have greatly benefited from the use of animal models. This narrative review comprehensively analyzes research on established rat model of shoulder contracture and new treatment approaches. This review evaluated existing literature on the available techniques for inducing contracture models, assessed these models, conducted pathological analyses, and explored their application in developing new treatment interventions. Our review highlights the usefulness of different rat shoulder contracture models, including external immobilization, internal immobilization, and intra-articular injection models, each with varying levels of success. Pathological analyses have demonstrated similarities to the human condition. The effective models have been instrumental in developing new treatment interventions, including recombinant human relaxin-2, platelet-rich plasma, collagenase clostridium histolyticum, and peroxisome proliferator-activated receptor-γ agonists. Therefore, rat shoulder contracture models serve as valuable tools for researchers to establish an effective animal model foundation for investigating the etiology and potential treatment.

1. Introduction

Shoulder contracture, specifically, adhesive capsulitis, is a common condition characterized by fibrosis, inflammation, and capsular contractures of the shoulder. The term “adhesive capsulitis” was originally coined by Julius S. Neviaser in 1945 to describe this pathological condition [1]. Shoulder contractures are classified as primary or secondary contractures. Primary shoulder contractures occur spontaneously without any recognized underlying causes or associated factors [2]. In contrast, secondary shoulder contractures develop as a consequence of trauma, surgery, or post-operative immobilization [3,4]. Patients with shoulder contracture experience discomfort, pain, and limited range of motion (ROM) [5]. The condition is characterized by progressive fibrosis, inflammation, neoangiogenesis, and neoinnervation of the shoulder capsule [6]. Despite ongoing research efforts, the exact underlying causes and mechanisms of shoulder contracture remain unclear [7].

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Patients with shoulder contracture can benefit from physiotherapy, including stretching [8–10], mobility exercises [11], physical manipulation [12], and medications (such as, non-steroidal anti-inflammatory drugs and steroid injections). In addition, hydrodilatation [13] and surgical interventions including, manipulation under anesthesia or arthroscopic capsular release, are effective [14]. However, novel treatment interventions are necessary to address the diverse needs of patients with shoulder contracture [15].

To elucidate the underlying mechanisms of shoulder contracture and develop novel therapeutic interventions, developing animal contracture models is essential. Given their anatomical similarities to humans, rats have been extensively used for developing shoulder contracture and rotator cuff repair models [16,17]. Various techniques have been used to establish rat shoulder contracture models. This narrative review summarizes research on these models and recommends new treatment interventions based on the cumulative findings.

2. Shoulder contracture models

Currently, three commonly used rat contracture models are available: the external immobilization (EX-IM), internal immobilization (IN-IM), and intra-articular injection (IA-IN) models.

The EX-IM model is a popular, non-invasive option. In particular, plaster-based EX-IM has been widely used. This method involves placing the rat's shoulder in adduction and internal rotation, followed by molding a plaster cast around the shoulder and chest areas. This cast is typically left in place for 4–6 weeks (Fig. 1; Table 1) [18–22]. The advantage of this model is that it is simple to implement, noninvasive, does not require incisions, and preserves the integrity of the shoulder joint. Therefore, we selected the EX-IM model (Fig. 1). However, variation in limb positioning between animals during immobilization and the loosening of plaster cast over time necessitating frequent remolding are major limitations [20].

The IN-IM model is another widely used shoulder contracture model developed by Kanno et al. [23]. Two plastic plates and flexible wires were used to fix the outer edge of the scapula to the humerus to immobilize the shoulder. Subsequent modifications by

Fig. 1. External immobilization model: molding the plaster around the shoulder and the chest [20].

Table 1
Different shoulder contracture models.

Contracture Model	Method	Advantage	Disadvantage
EX-IM model [18–22]	Immobilizing the rats shoulder in adduction and internal rotation by molding a plaster cast around the shoulder and chest area for a period of 4–6 weeks.	Simple, non-invasive, and no skin incision.	The limb position various, the plaster cast easily loosens over time, needs frequent remolding
IN-IM model [24–28]	Fixing the outer edge of the scapula to the humerus by plastic plates or polyester sutures for about 8 weeks.	Easy reproducibility	Surgical invasion and skin incision
IA-IN model [29]	Injecting adenovirus-TGF-β1 into the rat shoulder capsule	Easy reproducibility	Unclear safety and efficacy

EX-IM model: external immobilization model; IN-IM model: internal immobilization model; IA-IN model: intra-articular injection model.

Villa-Camacho et al. involved the use of two polyester sutures to tighten the humeral shaft and medial border of the scapula (Fig. 2; Table 1) [24–28]. Internal extra articular fixation was performed for approximately 8 weeks to immobilize the glenohumeral joints. Although this model offers easy reproducibility, it requires surgical intervention to establish internal fixation [23].

A recent study described another method for inducing shoulder contracture in rats via intra-articular injection (IA-IN). Specifically, ultrasound guidance was used to inject adenovirus-TGF-β1 into the rat shoulder capsule, resulting in the successful development of a primary shoulder contracture model. However, the safety and efficacy of this model remain unexplored [29].

Each model has its own advantages and disadvantages, and the selection of the model is based on the specific research goals.

3. Evaluation of shoulder contracture models

To assess the effectiveness of the shoulder contracture model, radiographs of the shoulder joint at the maximum passive rotation or abduction ROM are obtained, and a 10-g constant torque is applied to the humerus after detaching the shaft of the shoulder girdle from the trunk [23]. The ROM of abduction in the EX-IM model group was significantly lower than that in the control group [18–22], with a significant reduction in the measured mean abduction and rotation ROM in the group [23].

A device customized for measuring angle and stiffness in vivo has been reported in the literature. For instance, Villa-Camacho et al. utilized a sensor system consisting of a rotating axle, an arm clamp, and an orientation sensor with three degrees of freedom in addition to a reaction torque sensor to enable ROM and torque measurement [24,26–29]. The IN-IM group exhibited a significant decrease in the total ROM compared to baseline, with residual ROM limitations persisting for weeks after immobilization. Stiffness, measured as the area under the curve of the angle torque, increased after immobilization. Chen measured the stiffness of the rat shoulder joint using a customized torque measurement device that passively rotated the joint in both external and internal directions. The kinematic test revealed that, compared with the group, the adenovirus-TGF-β1-induced IA-IN model group required more torque at equivalent rotation angles [29].

Radiographic assessment is an efficient method for measuring ROM without using specialized equipments. However, a customized device with a sensor assembly is more accurate; therefore, preferred by many researchers. Although magnetic resonance imaging (MRI) and ultrasound are widely used to evaluate shoulder contracture in clinical practice, their application in the rat shoulder contracture model is limited. Future studies should explore the feasibility of these imaging modalities to enhance evaluation accuracy.

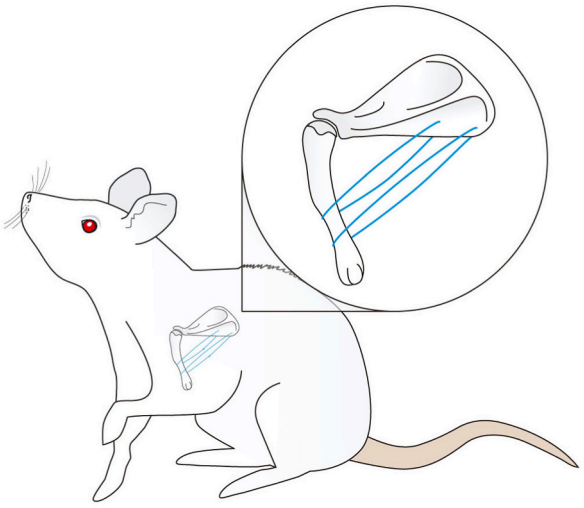

Fig. 2. Internal immobilization model: fixation of the scapular border and the humerus with braided polyester sutures [28].

4. Histologic and pathophysiologic evaluations

To assess the degree of adhesion via histological evaluation, hematoxylin–eosin (H&E) or Masson's trichrome staining was used to examine the synovium and capsule mostly in the axillary pouch. Kanno observed that the rat shoulder contracture model exhibited a shortened intraarticular synovial intima between the attachment site on the humeral neck and the inferior margin of the glenoid, with disappearance of the synovial fold, and the axillary pouch was covered by thick subsynovial tissue [23]. Kim et al. reported a decrease in subsynovial fat tissue and the synovial fold of the axillary recess, with capsule thickening and adherence to the bony cortex. Fibrosis in immobilized shoulders was detected using Masson's trichrome stain [18,20,22]. Okajima et al. reported fibrotic adhesion in the inferior glenohumeral joint (axillary pouch), with the surrounding tissue appearing denser, resulting in a tighter capsule with less joint space in the IN-IM group [24,26,27]. Feusi observed flattening of the synovial folds in the posterior synovial membrane [25]. Liu revealed adhesion of the subscapsular bursa to the hyperplastic synovium in the anterior capsule in the immobilization group [21]. Chen identified proliferation of the inflammatory cells and fibrocytes, neovascularization, and disruption of the fiber structure in the adenovirus-TGF-β1-induced IA-IN model group (Table 2) [29].

Immunohistochemical (IHC) staining has been routinely used to analyze the distribution of collagen and matrix proteins. IHC findings revealed increased expression of type III collagen in the synovium, particularly in the deep synovial subintimal area and spatium intermusculare, in the immobilization group [21,23]. Furthermore, IHC analysis revealed positive staining for Interleukin 6 (IL-6), Matrix Metallopeptidase 2 (MMP-2) and MMP-9 [18], and sections stained for fibronectin in the IN-IM group showed increased capsular thickness (Table 2) [24].

Taguchi reported abnormal staining on digital subtraction and thoracoacromial artery angiography in the immobilized shoulder joint [22].

In summary, the rat shoulder contracture models exhibited characteristic pathological changes, including a reduction in the length of the intra-articular synovial intima, synovial fold, and subsynovial fat tissue of the axillary recess and a thickened capsule that adhered to the bony cortex, resulting in decreased joint space (Fig. 3). IHC staining revealed an increased distribution of collagen and matrix proteins, whereas angiography indicated abnormal artery staining. These pathological variations are consistent with those observed in human shoulder contractures [27]. However, mechanical studies on this topic are scarce.

5. New treatment interventions in rat shoulder contracture models

Local delivery of recombinant human relaxin-2 (RLX) has been suggested as a promising biotherapeutic approach for alleviating shoulder contractures. RLX is a naturally occurring anti-fibrotic peptide hormone that induces growth and is upregulated during childbirth to increase tissue laxity [30,31]. In several animal models of fibrotic diseases, including those of pulmonary, cardiac, and hepatic fibrosis, RLX inhibits fibrogenesis and collagen upregulation through multiple mechanisms [32–34]. In vitro studies have demonstrated that recombinant RLX downregulates type I collagen and α-smooth muscle actin production in human fibroblast-like synoviocytes. Multiple intra-articular injections of RLX in the IN-IM rat model significantly improved the ROM of the immobilized shoulder, reduced fibrotic adhesions, and restored joint space, as revealed by histological assessment [24]. Furthermore, to overcome pharmacokinetic barriers, sustained-release RLX microparticles were designed, and a single injection of RLX-2-loaded microparticles successfully restored limited ROM and joint architecture in an IN-IM rat model. In addition, RLX receptor expression has been confirmed in human synovial joints [26], suggesting that local delivery of recombinant RLX may be a promising clinical approach to alleviate shoulder contracture associated with adhesive capsulitis.

Platelet-rich plasma (PRP) has emerged as a promising therapeutic option for shoulder contracture. PRP is a bioactive blood component rich in growth factors, which may influence tissue remodeling and stimulate healing [35]. In addition, the clinical application of PRP for adhesive capsulitis has been documented [36]. In an IN-IM rat model, intra-articular PRP injections demonstrated a reduction in posterior synovial membrane inflammation, without adverse effects [25].

Additionally, collagenase clostridium histolyticum (CCH), an enzymatic injection consisting of two synergistic collagenases, for treating Dupuytren's disease and Peyronie's disease has demonstrated substantial efficacy [13,37,38]. Clinical extra-articular CCH injections for shoulder contractures are safe and effective [39]. In rat models of shoulder contracture, subacromial injection of CCH improved the abduction ROM and decreased shoulder joint fibrosis. These studies support the potential therapeutic role of CCH in shoulder contracture [19].

Furthermore, peroxisome proliferator-activated receptor-γ (PPAR-γ) activation is a key mechanism for anti-inflammatory and anti-fibrotic effects, which also plays a role in the downregulation of physiological and pathological extracellular matrix reconstruction in connective tissues [40,41]. In vitro studies have demonstrated that PPAR-γ agonists can inhibit fibrosis induced by TGF-β1 in human skin fibroblasts. Additionally, in the intra-articular adenovirus-TGF-β1 injection shoulder contracture model, PPAR-γ agonists demonstrated positive therapeutic effects by inhibiting inflammation, reducing matrix protein production, and remodeling fibrous tissue structure. These findings suggest that PPAR-γ agonists could be a promising candidate for the treatment of shoulder contracture [29].

Finally, transcatheter arterial embolization has shown promise in shoulder contracture rat models by improving speed and ROM and reducing the number of blood vessels and inflammatory changes. However, further research is needed to fully understand its mechanisms [14,22].

Novel therapies such as recombinant RLX, PRP, CCH, PPAR-γ agonists, transcatheter arterial embolization, and other cutting-edge therapies offer novel potential therapeutic approaches for shoulder contracture in rat models. However, translating these findings into effective clinical therapies will require substantial time and rigorous investigation.

Table 2
Functional evaluation and pathology changes in different shoulder contracture models.

Studies	Contracture Models	Follow Ups (weeks)	Functional Evaluation	Histologic Changes
Kanno et al., 2010 [23]	IN-IM model Immobilized with two plastic plates, screws and flexible wires	8	Radiographs The abduction and rotation angle decreased	H&E staining: The length of the synovial intima shortened, synovial fold disappeared and the axillary pouch was occluded by thick subsynovial tissue IHC staining for type III collagen: the synovium, particularly the deep synovial subintimal area stained positively
Liu et al., 2011 [21]	EX-IM model Immobilized with plasters	4	NA	H&E staining: the hyperplastic synovium of the anterior capsule and adhesion of the subscaplular bursa. IHC staining for type III collagen and the picrosirius red staining: distributed at the synovium, subsynovium and extensively to the spatium intermusculare Enzyme-linked immunosorbent assay (ELISA): capsular content of type I and type III collagen increased
Villa-Camacho JC et al.,2015 [28]	IN-IM model Immobilized with two No. 2-0 braided polyester sutures	8	Customized device Rotational ROM decreased, rotational torque increased and joint stiffness increased	NA
Kim et al., 2016 [20]	EX-IM model Immobilized with plasters	6	Radiographs Abduction angle decreased	H&E staining: the synovial fold and subsynovial fat tissue disappeared, capsular thickened, and capsular adherence to bony cortex in the axillary recess Masson's trichrome staining: fibrosis was observed in the axillary recess
Okajima et al., 2018 [27]	IN-IM model Immobilized with two braided polyester sutures	8	Customized device A 63 % decrease in total rotational ROM; restriction (18 % of total ROM) was still apparent at 8 weeks of follow-up; an increase of 13.3 Nmm in total torque	H&E staining: capsular adhesions in the inferior aspect of the glenohumeral joint; the surrounding tissue appears to be denser; IHC staining for fibronectin: capsular thickness increased
Cho et al., 2019 [18]	EX-IM model Immobilized with plasters	3	Radiographs The abduction angle decreased after 1 week and 3 weeks immobilization	H&E staining: the synovial fold decreaed and subsynovial fat tissue disappeared, and capsular thickened in the axillary recess IHC analysis for IL-6, MMP-2, and MMP-9: the expression of all increased
Blessing et al., 2019 [24]	IN-IM model Immobilized with No. 2–0 Ethibond polyester suture	8	Customized device Rotational ROM decreased and rotational torque increased	H&E staining: lacked the separation between the capsule and the articular surface in the most inferior aspect of the glenohumeral joint and showed evidence of capsular adhesions IHC analysis for fibronectin: fibrotic tissue increased and capsular tissue thickened
Karahan et al., 2020 [19]	EX-IM model Immobilized with plasters	3	Radiographs Abduction angles decreased	H&E staining: the synovial fold, and sub-synovial fat tissue disappeared, lymphoplasmacytoid cells infiltrated.
Feusi et al., 2022 [25]	IN-IM model Immobilized with a 2-0 Fiber Wire	8	NA	H&E staining: hyperemia, atrophy of the synovial epithelium, flattened synovial folds, and fibrosis of the sub-synovium as well as hyperemia, angiogenesis, fibrosis, and edema in the fibrous capsule
Chen et al., 2021 [29]	IA-IN model Adenovirus TGF-β1 was injected by ultrasound guided into the capsule of shoulder joint	5	Customized device The rotational torque of the rat shoulder joint increased	H&E staining: proliferation of fibrocytes and inflammatory cells, neovascularization, and fiber structure disorder IHC analysis: vimentin and α-SMA's expression increased
Taguchi et al., 2021 [22]	EX-IM model Immobilized with plasters	6	NA	Angiography: abnormal shoulder staining H&E staining: decrease in the synovial fold and the subsynovial fat tissue, infiltration of inflammatory cells, proliferation of the synovial lining cells, and fibrosis of the joint capsule
R. Kirsch et al., 2022 [26]	IN-IM model Immobilized with No. 2–0 Ethibond polyester suture	8	Customized device Rotational ROM decreased and rotational torque increased	H&E staining: decreased synovial space, thickened synovial membrane, and fibrotic adhesions Masson's trichrome staining: fibrotic tissue presented in the axillary pouch and synovial membrane

EX-IM model: external immobilization model; IN-IM model: internal immobilization model; IA-IN model: intra-articular injection model; H&E:Hematoxylin-Eosin; IHC: immunohistochemistry; ELISA: enzyme-linked immunosorbent assay; NA: not evaluated.

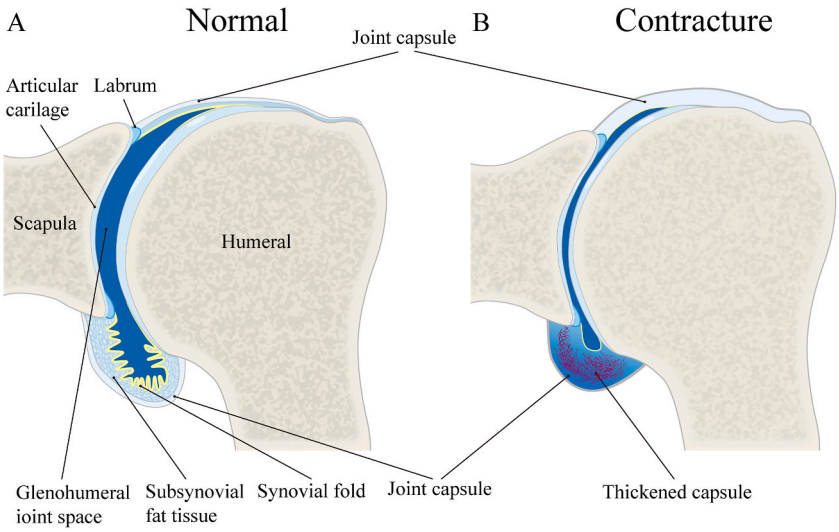

Fig. 3. Normal (A) and contracture (B) shoulder joint: the synovial fold of the axillary recess and the subsynovial fat tissue decreased; the fibrotic capsule thickened and adhered to the bony cortex with a decreased joint space in the contracture model [26].

6. Conclusions

Three rat shoulder contracture models, EX-IM, IN-IM, and IA-IN, were investigated. Radiography and custom-designed devices with sensor assemblies are commonly used to evaluate shoulder contracture. Histological assessment of the synovial and subsynovial fat tissues and the capsule of the axillary recess indicated fibrosis in the shoulder contracture model. Novel treatment strategies, such as RLX, PRP, and CCH, developed using rat shoulder contracture models, offer new potential approaches for the management of shoulder contracture. However, the translation and application of laboratory findings in clinical practice are often time-consuming and warrant further research. With in-depth research on the pathophysiology of shoulder contracture, future studies should focus on establishing more precise animal models for different stages of shoulder contracture onset. Furthermore, it is necessary to investigate novel and efficient treatments for the various phases of shoulder contracture.

Data availability statement

All data have been either provided or are otherwise publicly available, and the data will be made available on request.

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CRediT authorship contribution statement

Xiangnan Yuan: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization. **Shengwen Feng:** Formal analysis, Data curation. **Wanan Xiao:** Formal analysis, Data curation. **Jianjun Li:** Writing – original draft, Visualization, Supervision, Methodology, Investigation. **Yu He:** Investigation, Formal analysis. **Fenghua Zhou:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology. **Xueyong Liu:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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