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*Review Article*

# Role of Preclinical Arthritis Models for the Clinical Translation of Anti-Arthritic Therapeutics

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**Abstract**

The overcomplicated and elusive pathophysiology with unclear etiology, have been the main driving force of the medical scientists all over the world to develop a predictive, reliable, robust and reproducible simulation model of arthritis. This review highlights osteoarthritis and rheumatoid arthritis with distinct conditions pertaining to each type of disease. The advances in various *in vitro* and *in vivo* experimental models of osteoarthritis and rheumatoid arthritis have been presented along with their pros and cons for antiarthritic drug discovery and formulation development. Additionally, the ethical issues to be considered while selecting animal models and handling them have been covered briefly. The current status quo on clinical trials of antiarthritic therapeutic interventions has also been covered.

## Introduction

The term "Arthritis" is originated from Greek word "Arthron" meaning joint and Latin word "Itis" which translates to inflammation.[1] Arthritis is a collective term representing inflammatory diseases mainly affecting the joints and their surrounding tissues. It is one of the most ubiquitous inflammatory diseases, which wreaks havoc in mental, physical and economical aspects of patients' life.[2,3] The arthritis includes myriad of pathological conditions like rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout, infectious arthritis, fibromyalgia, juvenile arthritis, septic arthritis and psoriatic arthritis.[1,4] The most commonly occurring arthritis are the osteoarthritis (OA) and rheumatoid arthritis (RA).[5] The primary symptoms of both these form of arthritis are often confused with one another, which includes swelling, pain and stiffness of the joints.[6] Although the symptoms appear to be similar, RA is the resultant condition of an autoimmune attack, whereas OA develops with age due to degeneration of bones and surrounding tissues. In order to understand the pathogenesis of arthritis, it is imperative to be familiar with the typical mechanics of bone joint. Joint is the anatomical structure connecting two or more bones and it facilitates movement depending on the type. A typical joint consists of cartilage, synovial membrane, synovial fluid, ligaments, tendons, bursas and meniscus. Each of these components has specific functions.[7] Depending on the severity of the condition; arthritis may involve all these components of the joint.

OA is a chronic heterogeneous degenerative disease of joints, which predominantly occurs with age and most commonly in women.[8] It occurs due to gradual degeneration of cartilage tissues and slow erosion of ligaments tissues surrounding the joints, thus making the movement painful due to friction.[9] Along with the spine, the peripheral weight bearing joints mainly the knees, hands, hips are also gravely affected.[1] The OA affects the entire joints and other underlying subchondral tissues and synovium as opposed to the earlier theory of OA as a disease of articular cartilage alone.[10] The body develops new bone outgrowths called osteophytes in response to the damage in articular cartilage, thereby initiating a chronic inflammatory process with the release of cytokines and other inflammatory mediators.[11] The exact mechanism and etiology of OA is not clear, but the evidences over the time points towards the multitude of factors like cellular, mechanical and biochemical processes.[12,13]

RA is one of the most regnant chronic inflammatory autoimmune diseases, which adversely affect the joints, wreaking havoc in the lives of at least 0.5% to 1% of general population in the world.[14] The prevailing traits of the RA include stiffness of the joints, synovium inflammation and swelling as a result of increase in the synovial exudate, cartilage and bones erosion.[15,16] This ultimately leads to possible disability, shorten life expectancy and ominously impact the health of the suffering individuals.[17] The exact cause of RA is still not conspicuous, but the disease epidemiology has been attributed to the genetic and some exogenous factors which acts as 'triggers' in the genetically predisposed individuals.[18,19] Certain autoantibodies

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specific to RA are involved in the pathophysiology, which gets attached to citrullinated proteins that are present in RA manifestation. Few examples of these autoantibodies include anti-cyclic citrullinated peptide antibodies (ACPA), Anti-carbamylated protein antibodies (anti-CarP antibodies). Autoimmunity starts at molecular and cellular level as a result of the presence of these autoantibodies in certain genetically predisposed individuals' before the onset of clinical arthritis.[15]

Over the decades, numerous diagnostic and treatment strategies have been adopted to counteract both these form of arthritis in the form of therapeutics, surgical techniques, physiotherapy including certain changes in the lifestyle.[20,21] Among the therapeutics, the analgesics, steroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and biologics like mono-clonal antibodies, TNF inhibitors have been widely incorporated in the treatment regimen of arthritis.[22,23] However, the conventional treatment options bear several setbacks such as low bioavailability, gastric irritation, bleeding, renal toxicity, myelosuppression and cardiac-related complications.[24-26] Gradually, the medical and pharmaceutical scientists have incorporated the novel nanomedicines for the management of arthritis with the objectives to provide precise medications reducing the adverse effects associated with the conventional therapy. Multiple nano-based drug delivery systems have been explored with their ability to increase the bioavailability, solubility and targeted delivery of the drug by tuning their surface characteristics, thus reducing the systemic adverse effects.[27-30] Like many other chronic diseases, the exact scenario regarding the causes, etiology and pathogenesis of arthritis is still unclear, hence, necessitating the simulation of these conditions so that potential therapeutic regimen can be developed.[15,31] This can be achieved by developing *in vitro* and *in vivo* models of arthritis, which is not an easy task considering the maze of human's physiology. The mounting literature available on anti-arthritis study over the years is the evidence of the time, efforts and attempts made to replicate this debilitating disease into models using different experimental animals.

Although, there are many literatures discussing the models of arthritis, but none has comprehensively reviewed the advances of both *in vitro* and *in vivo* experimental models of OA and RA in a single paper. The ethical concerns to be considered and followed while using animal models have also been discussed with the possible alternatives for refinement of animal usage. The current status quo on clinical trials of antiarthritic therapeutic interventions has also been covered.

## *In Vitro* Experimental Arthritis Models

An array of *in vitro* models has been established in order to study the pathogenesis of arthritis and also to determine the efficacy of the potential anti-arthritic medications. Depending on the type of the research question and intention of the researcher, suitable models are selected that can serve the purpose and fulfil the requirements at least to some extent, if not completely. The growing concern over the involvement of animals in the biomedical experiments has led the researchers to look for alternatives laced with ethical values, which may serve the purpose of the research question. *In vitro* models of the joint are required to interpret the molecular pathways and mechanism of joint pathophysiology.[32] Over the past decades, numerous *in vitro* models for joint diseases particularly arthritis, are being used and they can be broadly categorised into two-dimensional (2D) cell culture models, three-dimensional (3D) cellular models and microfluidic organ on a chip system, which are further sub-classified. Typical *in vitro* models of arthritis are represented in Figure 1.

### *Two-dimensional (2D) cell culture models*

2D cellular models are the simplest primary *in vitro* models available for arthritis. It involves culturing of one or more primary cell lines in a flat surface preferably of polystyrene material containing media of required growth factors and nutrients essential for the multiplication and proliferation of the cells.[33] The monolayer model involves the culture of only one cell line, whereas co-culture involves the culture of two or more cell lines.[34]

#### *Monolayer culture*

2D monolayer cell culture model tends to be the most economical, simplified *in vitro* arthritis model with flexible experimental parameters giving high-throughput, reliable and fast outcomes.[33] In this model, either harvested primary cells or immortalized cells from different sources like human, bovine, murine are cultured in a polystyrene flat surface with equal distribution of culture media containing growth factors and nutrients. Articular cartilage cells,

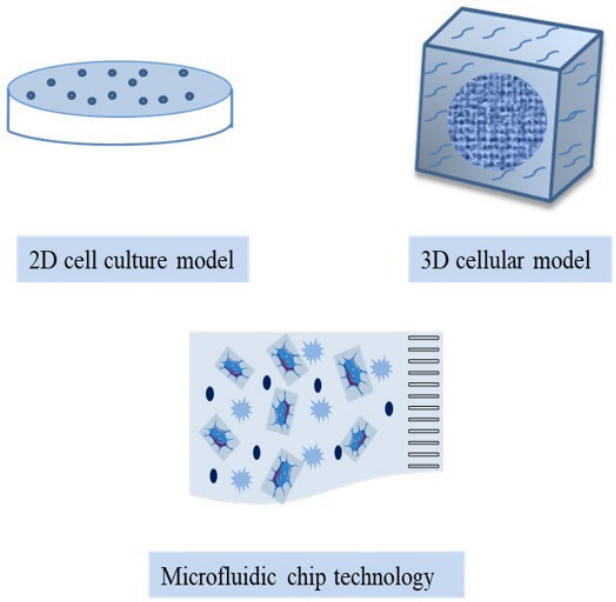


**Figure 1**. A typical pictorial representation of *in vitro* arthritis models.

synovial cells, chondrocytes cells, stem cells are some of the most commonly used cell lines for 2D culture models.[35] The types of cells for 2D cellular models are enumerated in Table 1. Since monolayer cultures deals with culturing single cell line, it avoids the problems associated with culturing multiple cell lines at different conditions and hence it can be produced at large scale. As the sources of primary cells are hard to obtain and have problems of sub-culturing owing to their de-differentiation and loss of their unique phenotype, they are more expensive than cell lines. Despite the risk of alteration in cellular morphology, cell differentiation and proliferation capacity, alteration at genetic level, 2D monolayer cell cultures are used for studies requiring rapid *in vitro* cell expansion.[36] These models are also useful for investigating the optimal concentration, efficacy and efficiency of antiarthritic therapeutics along with the disease related gene expression profile analysis.[37] Although these models are oversimplified, they do give meaningful and reliable insights to our understanding of the events persisting in joint diseases. But, the information is only limited to certain isolated events within a specific tissue types like cartilage, which undergoes changes in their phenotype once in a monolayer culture environment, arising inter-experimental variability. Also, a large sample of cartilage is required to ensure sufficient numbers of cells for a reliable experiment.[35] Traditionally, cells in monoculture grow on a flat surface in glass or polystyrene flasks and therefore do not allow for growth in all directions, simulating the natural 3D *in vivo* environment.[38]

### *2D co-culture*

As contrast to monolayer culture of a single cell line, in co-culture, two or more cell lines are cultured together. Hence, *2D Co*-culture allows cell-cell interactions investigation in a shared environment. For example, a model where chondrocytes are incubated with osteoblasts has been useful in studying the effects of chondroprotection (slowing down of cartilage degradation) in bone remodelling. It also helps to maintain cellular phenotype and physiology by paracrine signalling.[39] Another example is, where chondrocytes incubated together with synoviocytes are stimulated by proinflammatory cytokines to determine the cross-talk between cells through intercellular calcium and paracrine signalling by maintaining homeostasis of articular chondrocytes. These models can also be used to investigate multiple experimental treatments at once like monolayer models and therefore have the ability to generate extensive data on joint pathological mechanisms.[40,41] Despite several advantages provided by culturing multiple varieties of cells together, this *in vitro* model suffers from limitations like altered cell morphology and inability to investigate direct cell-ECM interactions. It is also troublesome and expensive to maintain different cellular environments at the same time. Another drawback lies in the fact that only one or two tissue components can be isolated at a time which fails to understand certain events like communication network among different components of joint, which helps in regulating and maintaining a healthy joint, and so isolation of specific joint components circumvents this communication.[42]

### *3D cellular in vitro models*

Since, 2D cellular models are associated with many limitations; there arises a need for more reliable, reproducible and robust *in vitro* cellular culture approach with economic viability and ability to investigate the cell-matrix interaction in disease pathology. This brings us to better alternative i.e. 3D cellular models, which is suitable

**Table 1.** Types of cells used in 2D cellular models.

| Types of cells | Uses | Disadvantages | Ref. |
|---|---|---|---|
| Articular cartilage cell lines | To detect specific pathological signatures via immunohistochemical and biochemical techniques | Descriptive data limits possible mechanical insights<br>Difficulty to define the positive and negative controls | 39 |
| Primary articular chondrocytes cell lines | Because of their ability to dedifferentiate, they can be used as a suitable model for OA | Chondrocytes lose their molecular signature outside joint environment<br>Cells are difficult to obtain, transfect, hence limiting possible alteration of target gene expression<br>Ethical problems in obtaining human chondrocytes | 39 |
| Immortalized clonal cell lines | To study the factors and signalling pathways that influence cell behaviour during the processes of chondrogenesis and chondrocyte differentiation | The potential to reliably substitute primary chondrocytes is not clear | 43, 44 |
| Human articular cartilage immortal cell lines | To study cartilage physiology and cartilage anabolic and catabolic processes | The potential to reliably substitute primary chondrocytes is not clear | 45 |
| Mesenchymal cell lines | To give multiple connective tissue lineages, including cartilage and bone | Defining optimal cell population is difficult<br>Challenges in surgical cell transfer | 46-48 |
| Synovial cell lines | To elucidate the role of the synovium in OA and to identify potential mechanisms relevant to the disease | Replicating *in vivo* response is difficult | 48 |

for a broad range of applications due to their ability to maintain the cell phenotypes such as articular cartilage chondrocytes.[33,34] The 3D approaches circumvents some of the shortcomings of above mentioned two-dimensional systems as these models facilitate cell–cell, cell–matrix interactions; cell differentiation, proliferation, and migration at the same time maintaining the fate of the cell as well, due to their physiological 3D structure. The development of 3D approach encompassing different types of joint components like synovial membrane, the osteogenic and chondrogenic parts are necessary to mimic the structural features of the joint in order to simulate the pathogenesis of arthritis.[37] Currently existing 3D cellular models are tissue explant based, hydrogel based (with scaffold), and pellet based (without scaffold), have been discussed in this section briefly.

### *Tissue explant models*

As the name suggest, the explants used in these models are obtained from *in vivo* tissue of either animal or human source depending on the feasibility and ethical considerations. The derived tissue explants are then cultured and maintained in 3D environment. It allows for *in vitro* manipulation like 2D models with maintaining the tissue viability unlike in 2D models, where cell alterations take place.[49] Explant models enables investigation of compressive overload on articular cartilage, thereby, providing insight into the impact of cartilage loading in disease progression. The osteochondral explant models may be used to investigate the relationship between articular cartilage and the underlying subchondral bone tissue. For example, in a study by Byron *et al.*,[50] cartilage explants challenged with IL-1β produced an elevated level of TNF-α compared to controls, whereas TNF-α secretion was unchanged in osteochondral explant, which suggests the presence of bone or synovium may reduce TNF-α expression and imparting the importance of considering all joint tissues in the study of OA pathophysiology.[50]

Undoubtedly, the explant-based models provide advantages over 2D cellular models. Some major drawbacks are also persistent with this model, for example, only few number of explants can be derived from a single donor and the instances of intra donor variability between samples is prevalent.[51] Another major drawback is the tendency of the explants to die around the surgical tissue edges.[35] The complication arises in long-term studies as the extracellular matrix composition and mechanical properties of the tissue explants starts changing over time. Although the tissue explants from animal source are relatively easy to obtain, the explants originating from human source are difficult due to ethical concerns and they are highly expensive.[32]

### *Hydrogel based 3D model*

This 3D model uses a matrix system called as scaffold that corresponds to the natural tissue properties and hence closely simulates the *in vivo* condition. These matrix or scaffolds not only give the three-dimensional support but also maintains cellular growth and proliferation, where the matrix elasticity, biochemistry and micro-architecture can be tuned.[52] Hydrogels are the most abundantly used tissue scaffold owing to their high-water content and tuneable mechanical and physicochemical properties. Other than this, certain biopolymers such as alginate, agarose, chitosan and hyaluronic acid have been applied to grow chondrocytes, mimicking osteoblasts and articular cartilage for modelling the osteochondral plate.[53] Hydrogels can be of biological and synthetic origin. The biological hydrogels are derived from natural resources. They have large water component and are preferred because of their likeliness to ECM biodegradability, biocompatibility, bioactivity, modifiability, porosity and low immunogenicity. Some biological based hydrogels can be constructed by 3D printing, which eliminates the hazardous process and the potential risks of crosslinking with toxic reagents. But the biological hydrogels suffer from inter-batch variation during manufacturing. Just like in case of explants, the source of the hydrogels may affect the properties of the gel.[54]

Synthetic hydrogels are basically derived from polymers and has advantage over its biological counterparts in the fact that they do not originate from finite source, which reduces variability among manufactured products. Synthetic hydrogels can also be finely tuned via chemical modification, such as the inclusion of chondrogenic molecules, which are delivered to hydrogel embedded cells.[55,56] It has been reported that the synthetic hydrogels derived from PEG di-methacrylate are able to be integrated into a mechanical loading system. This integration directs human mesenchymal stem cell differentiation into articular cartilage, calcified cartilage and subchondral bone tissues.[57]

Although hydrogels provide a wide range of materials compatible for *in vitro* modelling of arthritis, the composite components of the hydrogels must be considered properly. As the hydrogels are comprised of materials that are not exactly similar to those found in the natural ECM and hence may cause alteration in cell behaviour. The large water content of hydrogels also poses problem in some occasions as like in mimicking subchondral bone having very little *in vivo* ECM water content.[33]

### *Pellet based 3D cellular models*

In this model, cell pellets like chondrocytes and osteocytes together are centrifuged in multi-well plates or conical tubes using bioreactors. The cells are clumped together to form cluster of tissue-like pellets (up to 5mm size) after certain incubation time, thereby, producing 3D aspect to the culture system. Three-dimensional cellular pellet model overcome few of the disadvantages of 2D monolayer cultures as they maintain a particular structure, while facilitating cellular growth in all dimensions. These pellets can provide insights into cell-to-cell and cell-to-ECM relationships as they mimic articular tissue as a whole.[58-60] The main disadvantage of the pellet culture models is that they exhibit a depletion in cell proliferation.

The nutrients in the culture have difficulty in penetrating the pellet, which ultimately result in starvation of cells and hence cell death. Also, the culture media requires adequate supplementation of high amount of growth factors and chondrogenic stabilizers, thus these are not cost effective compared to monolayer culture.[60,61]

### *Microfluidic organ on a chip based approach*

The discussed 2D and 3D cellular models (Table 2) are static in nature. Although they have some *in vivo* similarity aspects in their system, they still are devoid of mechanical stimuli like shear stress, tension, compression that cells naturally experience *in vivo*.[32] This issue has led to a more challenging and advanced sophisticated system of microfluidic technology, where organs are bio fabricated on microfluidic chips. This technology allows the dynamic flow conditions and mechanical stimulation that may aid in the making of more predictive human synovial joint models. Till now, a number of 3D culture techniques including 3D human organoids, human organ-on-a-chip and bio fabricated tissue-like structures have been explored to model physiological and pathological human conditions.[62,63] These few predicted microfluidic culture approaches have been reported to at least partially reflect the physiology of the joint structure mimicking either subchondral bone, articular cartilage and/ or both.[64,65] Microfluidic systems can be connected with each other to form multicompartment microfluidic devices. This multicompartment organization is ideal for the design of *in vitro* models of the joint, a multi-tissue organ, where cells are exposed to different types of mechanical stimuli. However, the principal challenge when connecting different microfluidic devices involves the requirement for a common media, or blood substitute, suitable for each

**Table 2.** Efficiency of *in vitro* cellular models for the development of anti-arthritic interventions.

| Type of arthritis | Type of *in vitro* models | Type of cells/tissues used | Therapeutic intervention | Findings | Ref. |
|---|---|---|---|---|---|
| OA | 2D monolayer | HIG-82 cell line (rabbit synoviocytes) | Liposome-encapsulated fish oil protein-tagged gold nanoparticles | Sustained release of fish oil protein in synovial fluid was exhibited | 66 |
| OA | 2D monolayer | Human primary chondrocytes | Curcumin nanoparticles | The nanoparticles exhibited anti-catabolic and anti-inflammatory effect in human chondrocytes *in vitro* | 67 |
| RA | 3D cellular | Tri-culture of synovial fibroblasts (SFs), LPS-stimulated macrophages and a primary chondrocyte-based tissue engineered construct | Celecoxib | The 3D model developed was able to respond to the therapeutic effects of Celecoxib by reversing cartilage damage. | 68 |
| RA | 2D monolayer | The human synovial cell line SW982 | Fluvastatin | Fluvastatin induced apoptosis in a dose- and time-dependent manner in TNFα -stimulated SW982 human synovial cells | 69 |
| OA, RA | 2D monolayer | Isolated human chondrocytes | Celecoxib and Diclofenac | Both drugs reduced 3H-tymidine incorporation of chondrocytes and induced apoptosis in a dose-dependent manner | 70 |
| OA | 2D monolayer and co-culture | Human bone marrow-derived mesenchymal stromal cells, Osteochondral explant | Kartogenin | KGN enhances proliferation and chondrogenic differentiation of hBMSCs. | 71 |
| OA | 2D monolayer | Bovine chondrocytes | Hyaluronic acid loaded Cerium oxide nanoparticles | The chondrocyte inhibition decreased with supplementation of cerium oxide nanoparticles | 72 |
| OA | 3D engineered | Cartilaginous tissue | Celecoxib | Inhibition of nitric oxide and prostaglandin E2 production, as well as down-regulation of MMP-1 and MMP-3 expression. | 73 |
| OA | 2D monolayer | Human chondrocytes | Chrysin | CH inhibited inflammatory mediator expression via HMGB1 suppression | 74 |
| OA | 2D cellular | Rat chondrocytes | Hyaluronic acid/chitosan nanoparticles loaded with curcuminoid | The formulation decreased chondrocyte apoptosis in rats with knee OA through repression of the NF-κB pathway | 75 |

tissue of the interacting system.[32] Despite their advances and opportunities for translational studies and drug testing, microfluidic systems still have some limitations. So far, microfluidic systems are more challenging to operate and control than static systems. Some organ functions, such as cognition on the brain and mechanical function in bone cannot be readily modelled and they are difficult to adapt to high-throughput screening and hence are difficult to standardize and scale up.[37]

## *In Vivo* Experimental Arthritis Models

Animal models of human diseases are developed with an objective to simplify the elusive and exasperating phenomena and processes. Animal models generally involve a homogenous group of animals having an inherited, naturally acquired, or experimentally induced biological process that resembles the disease in humans and the condition is amenable to scientific investigation.[76] Over the years, the animal models of arthritis have played an indispensable role in providing insights into the underlying disease pathogenesis. The animal models help in identifying new targets and biological markers for drug molecules and thus to develop new therapeutic and diagnostic interventions. The animal models must have similar features to the human arthritis pathogenesis in order to predict the efficacy of potential therapeutic agents in humans. The models must be reliable and reproducible in terms of mechanisms and outcomes.[77] In order to select an appropriate animal model, it is extremely important to critically analyse and understand the clinical relevancy of research problem under investigation and the hypothesis being tested.[78] There are mainly three criteria which help in choosing suitable animal models as suggested by Pritzker *et al.*[76] which are represented in Figure 2.

In this section, various experimental animal models of osteoarthritis and rheumatoid arthritis prevailing in the literature and/or under investigation in the arthritis-based research are briefly discussed.

### *Osteoarthritis animal models*

There are several established OA animal models. Each of them has their own pros and cons and hence none model is a gold standard. The OA animal models can be broadly classified into experimentally induced models and spontaneous models, which are further classified (Figure 3), and the sub classifications are discussed in this section.

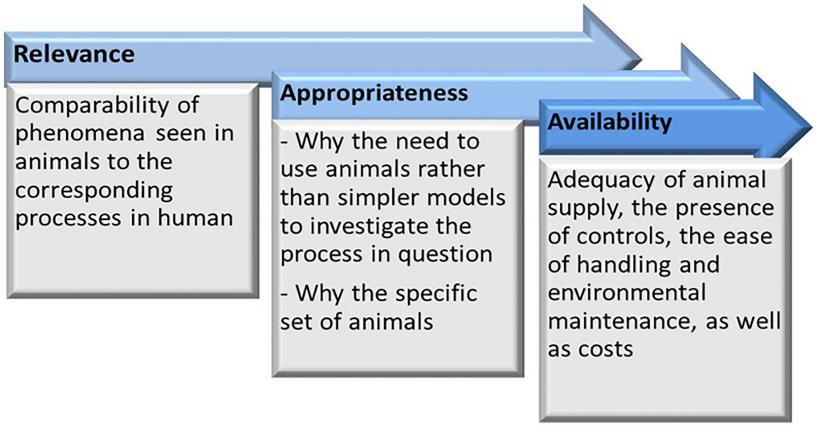


**Figure 2.** Criteria for selection of suitable animal models.

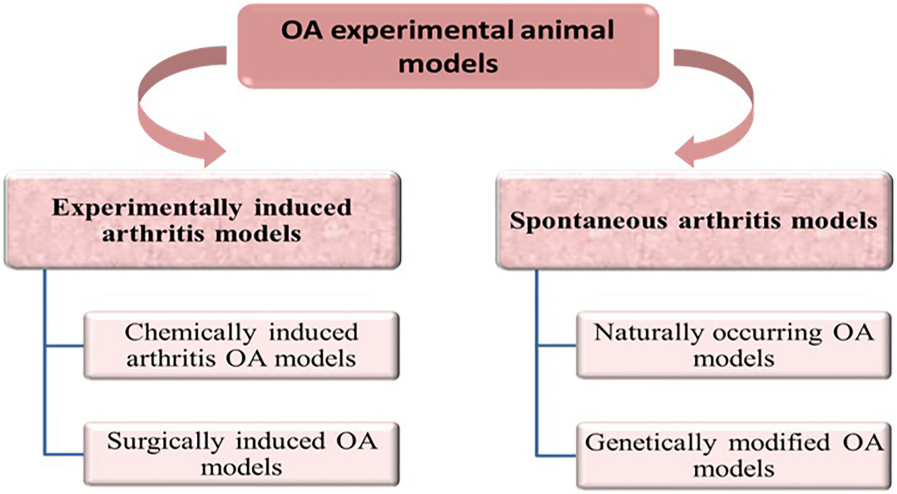


**Figure 3.** Osteoarthritis experimental animal models classes.

OA animal models are developed using both large and small animals. Small animal like the mouse, rat, guinea pig, and rabbits are used whereas, the large animal models include the goat, canine, sheep, and horse. The choice of each animal model depends on several factors like nature of experiment, time period, husbandry costs, outcome measurements, and ease of handling. The time duration needed to complete the experiment depends on the skeletal maturation of each animal, which is the time taken for each animal to reach skeletal maturity and, as a consequence develop OA.[79]

### *Experimentally induced OA models*

Experimentally induced models refer to the models where OA diseases (or OA like features) have been induced either chemically or surgically.

### *Chemically induced OA models*

A chemically-induced model of arthritis is one of the most cost-effective, reproducible and robust ways of simulating OA in laboratory animals like mice and rats.[80] It is because of that these models involve inducing OA with the help of certain chemicals. The chemical is injected directly into knee joints that will in turn manifest various inflammatory reactions and cause pain at the site. Hence, these models can be used to investigate the efficacy of new therapeutics by their ability to alleviate the pain. Sodium monoiodoacetate, quinolone, papain, and collagenase are some of the chemicals employed to induce OA in animals. The main advantage of these models is that they induce OA by eliminating the need for surgery and thus avoiding the possible surgical infection in some animals.[81]

The Monoiodoacetate (MIA) model is one of the most commonly used chemically induced OA model. This model mainly targets on the pain aspect of OA pathophysiology; hence this model is ideal for assessing OA related pain and peripheral neuropathy. This model was first demonstrated by Kalbhen in chickens. In this model, Sodium monoiodoacetate (0.5-1 mg) is injected intra-articularly into the joint of investigated animals. It leads to the reduction of chondrocytes number by disrupting the chondrocyte homeostasis by inhibiting glycolysis. It ultimately leads to the cartilage degeneration and subchondral bone loss. This chemical is mostly injected into the hip, knee, and ankle joints of small laboratory animals like rats, mice, and guinea pigs.[82,83] In this model, the OA pathology and OA pain typically develops within 1–2 weeks in a dose-dependent manner. The cartilage lesions are observed mainly in the medial tibial plateau upon histological analysis as early as 1–3 days after injection. The peripheral nerve inflammation signs start observing at 3$^{rd}$ day and upregulation remains at day 14.[84-86] Generally at the final day of experiment, the subject animals are anesthetized and euthanized following which knee joints are removed and fixated for histological examination.[87] The parameters involve the degree of cartilage degeneration, which is scored mostly using the Osteoarthritis Research Society International (OARSI) scoring system described by Pritzker *et al.*[88] and the score ranges from 0 to 24 with higher values representing more advanced cartilage degeneration.[88] Also, the body weight, paw withdrawal test, spleen weight along with knee joint thickness and X-ray radiography imaging of articular cartilage are evaluated as parameters of disease and treatment outcomes.[89] As evident from the available literature, MIA induced experimental OA model is the most persistently employed model to understand the progress of osteoarthritis and its potential therapeutic intervention to combat this debilitating condition.

### *Surgically induced OA models*

These models involve invasive surgical procedures in order to produce joint instability in animals to mimic the post-traumatic osteoarthritis. This is because the certain section of the population (like athletes) develops OA in early phase of their life due to traumatic joint injury despite OA being considered to develop with aging in elderly population.[90] These models are utilised in mice, rats, rabbits, dogs and sheep.[83] These invasive experimental OA models recapitulate most of the histopathological features of human OA causing joint instability, intra-articular inflammation and altering the load-bearing of the joints, which ultimately leads to post-traumatic OA (PTOA).[80] Medial meniscal tear, partial meniscectomy, total meniscectomy, anterior cruciate ligament transection (ACLT), articular groove, trans articular impact and ovariectomy are some of the invasive procedures utilised to induce OA in laboratory animals.[81] These surgical procedures are applied single or in combination depending on the nature of the study and desired disease progression features. Out of these, the most predominantly utilised model is ACLT for studying OA *in vivo*.

The ACLT model involves the injury to the anterior cruciate ligament of the experimental animal, which causes joint destabilisation subsequently leading to PTOA and articular cartilage degradation. Technically, the animal is first anesthetised followed by lateral incision of the skin of the right knee joint, which exposes the joint capsule. Then the ACL is transected using a surgical scalpel. The anterior drawer test is used to investigate the appropriateness of this procedure keeping the ACL-intact left knee joint as the control joint. This model is suitable for evaluating bone resorption inhibitors as potential DMARDs.[91] In this model, the animals are euthanized at 9 weeks after ACLT and stifle joint samples are collected for evaluation. Then the collected joints are evaluated for (a) macroscopic analysis looking for cartilage fibrillation and erosion, then the OA are scored using OARSI scoring system; (b) histological analysis where the changes in femoral condyles are observed and scored; (c) immunohistochemical analysis of articular cartilage for the expression of matrix metalloproteinases (MMPs).[92] ACLT is either generally performed single or in conjunction with other surgical methods like PCLT or medial and/ or lateral

meniscectomy in mice and rats.[75] The main advantage of this model is the slow development of OA lesions, hence enabling to study the disease progress thoroughly. Another surgically induced model is destabilization of the medial meniscus (DMM), a well-established and widely used surgically induced model of OA, usually studied for target validation investigations on genetically modified animals and evaluation of the roles of molecules and enzymes in OA pathophysiology *in vivo.*[93]

### *Spontaneous OA models*

Spontaneous animal models of OA mimic the slow progression of the human OA pathophysiology and thus are commonly employed to study the primary OA. There are two categories of spontaneous OA models namely, (A) naturally occurring OA models and (B) genetically modified OA models.[33]

### *Naturally occurring OA models*

There are certain strains of laboratory animals such as guinea pigs, rabbits, Syrian hamsters, sheep, dogs and horses that develop OA spontaneously.[81] One such example is the Dunkin Hartley guinea pig, which develops symmetrical OA lesions and joint changes early at the age of 3 months typically at medial tibial plateau. It ultimately leads to total medial degeneration at the knee joints by the age of 12 months to 24 months.[94] This model is the closest representation of the primary OA in humans, hence it is the most commonly utilised model to simulate the naturally occurring OA. In this model, the pathophysiological changes of the joint are not as a result of pain or nociception rather is related to age and body weight.[95] Besides the Dunkin Hartley guinea pig model, there are a few strains of mouse such as STR/ort and C57BL/6 strains, exhibits different OA vulnerability and develop spontaneous idiopathic OA. Whereas, certain strains like the CBA mice, are considered to have a resistance in developing spontaneous OA. These strains of mouse may be the effective controls for *in vivo* studies of OA.[96] The studies are assessed by histological and immunohistochemical analysis.

### *Genetically modified OA models*

Genetically modified animal models are becoming extremely popular in pharmaceutical research notably in OA studies. These animal models have the ability to genetically modify and breed to specific strains susceptible particularly to OA. Mice are the most commonly put through genetically modified breeding and these transgenic mice have played an enormous role in understanding the contribution of specific gene to the pathogenesis of OA.[80,97] The scientists have already sequenced the entire genome of mouse, thus making it relatively simpler to breed these animals with modified genome to study the genotype-phenotype correlations.[33] These models enable the investigation of the possible specific genetic influences associated with the articular cartilage inflammation, degradation and subchondral bone.[33,98] However, there is a disadvantage while developing therapeutic intervention targeting the specific genes, since these models does not account for other contributing genes influencing the OA pathogenesis. This may reduce the translatability of animal study results to clinical trials.[78] Some examples of genetically modified animals include the STR/ort mice, which exhibit increased oxidative stress inducing OA.[81,99] Mainly three proteases involved in OA progression are targeted while developing these transgenic mice. These three proteases, cathepsin K, MMP-13, ADAMTS-5 are responsible for degradation of major macromolecules cartilage.[100] Another genetically modified OA animal model is Zebrafish model, which exhibits the phenotypic features of OA including loss of articular cartilage, reduced joint mobility and formation of bony spurs. One such example is the transgenic *col10a1* reporter line, which allows the easy identification of hypertrophic chondrocytes that may play a role in the development of OA. Therefore, this model has been adopted for functional studies of genes that play a role in OA disease progression.[101,102]

The significance of *in vivo* experimental animal models in the biomedical research associated with OA is unquestionable. However, there is not a gold standard model despite of having overwhelmingly many models to choose from. Therefore, researchers have adopted the models in combination to produce desirable results. The efficiency of these experimental animal models has been presented in Table 3.

## ***Rheumatoid arthritis (RA) animal models***

The complex pathology of RA includes inflammation, autoimmunity and immunological tolerance. Several methods are used to induce this disease in experimental animals. The pain aspect of the RA is the primary concern, which is gravely affecting the suffering individuals physically and psychologically. The other aspects of disease pathology-related models also have immense role in understanding disease progression and finding effective therapeutics.[123] A wide range of animal species are used for developing RA animal models. However, the arts and mice are mostly preferred owing to their attributes like low cost, ease of handling, homogeneity of the genetic background.[124]

### *Experimental animal models dealing with pain aspect of RA/nociception models*

*A) Acute non-immunological formaldehyde-induced Paw edema test*

The formaldehyde induced paw edema test in mice or rat is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. In this model, acute non-immunological arthritis is induced by sub plantar injection of 0.1 ml formaldehyde (2% v/v) into the right hind paw of the animals at 30 min after the drug administration of day 1. Then the inducer dose is repeated on day 3 to sustain the inflammation during the course of study. The treatment course generally continues for 10 days. The arthritis is

**Table 3.** Few applications of *in vivo* OA models.

| Types of *in vivo* OA model | Therapeutics | Effects | Ref. |
|---|---|---|---|
| Dog OA model (closed surgery) | Licofelone | Reduced calcified cartilage and subchondral bone remodeling/ resorption, which may have contributed to the efficacy of the treatment | 105 |
| Dog ACL model | Tenidap | Reduced the progression of the disease | 106 |
| Meniscectomy sheep model | Diacerein | Improved cartilage and subchondral bone parameters in the lesional areas | 107 |
| Dog ACL open surgery | Doxycycline | Reduced the levels of active collagenase and gelatinase and the synthesis of iNOS | 108 |
| Hemi-meniscectomy Rabbit OA model | Sodium hyaluronate | Improved weight bearing and cartilage integrity in contrast to the untreated animals | 109 |
| Cruciate deficiency rabbit OA model | γ-cyclodextrin polysulfate | Reduction in cartilage scores and osteophytes with the higher doses | 110 |
| Hemi-meniscectomy rabbit OA model | Glycosaminoglycan | Reduced OA lesions in a dose-dependent manner | 111 |
| ACL rabbit OA model | Resveratrol | Reduction in cartilage destruction | 112 |
| Cruciate-deficient rabbit OA model | PD 198306 | Reduction in cartilage lesions, osteophyte size and synovial hyperplasia | 113 |
| Cruciate-deficient rabbit OA model | Benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone | Reduced cartilage degradation, while chondrocytes number increased | 114 |
| Rat meniscectomy model | Diacerein | Prevented the development of OA structural changes and reduced joint stiffness. | 115 |
| Spontaneous And the Collagenase-induced knee OA mouse models | Pralnacasan | Reduced the histological scores of lesions and the urinary marker of collagen degradation dose dependently | 116 |
| Meniscectomy Guinea pig OA model | Pioglitazone | Reduced OA cartilage lesions and decreased the production of MMP-13 and IL-1b, which are key mediators of cartilage catabolism | 117 |
| Dunkin Hartley guinea pig spontaneous OA Model | Doxycycline | Partial protection from the loss of cartilage on the medial tibial plateau | 118 |
| Rat ACL model | Alendronate | Exhibited chondroprotective effects with inhibition of osteophyte formation and reduction in cartilage degradation biomarkers level | 119 |
| Spontaneous guinea pig model | Risedronate | Slowed the progression of OA by reducing the severity of cartilage lesions and limiting osteophyte formation | 120 |
| Rat meniscectomy model | FGF18 | Simulated chondrogenesis and cartilage Repair | 121 |
| MIA rat model | Cilostazol | Demonstrated protective effect against cartilage degradation by reducing cartilage surface irregularities, matrix loss, chondrocyte apoptosis and cartilage iNOS expression | 122 |
| Spontaneous OA STR/ort mice | Glucosamine sulfate or hydrochloride | It delayed the appearance of OA changes in the mice | 123 |
| ACL rat model | Glucosamine sulfate or hydrochloride | Reduced cartilage damage by maintaining proteoglycans and inhibiting type II collagen degradation as well as by enhancing type II collagen synthesis in cartilage | 124 |

assessed by measuring the mean increase in paw volume using a digital Vernier calliper or Plethysmometer. The percentage inhibition of paw edema is calculated using the following equation:

$$\%Inhibition = \frac{V_c - V_t}{V_c} \times 100 \quad \text{Eq. (1)}$$

Where, $V_c$ is the paw volume of control group and $V_t$ is the paw volume of treatment group.[125]

*B) Acute non-immunological carrageenan-induced paw edema model*

The carrageenan-induced mouse and/or rat paw edema model deals with the pain and inflammation aspect of RA. Carrageenan is a substance that stimulates the release of inflammatory and proinflammatory mediators, such as tachykinins, histamine, bradykinin, reactive oxygen and nitrogen species.[126] In this model, the animals are injected

with a certain concentration (usually 1%) of carrageenan in one hind footpad, usually at 30 minutes to 60 minutes after they are treated with the test compound while saline is used as a control on other footpad. Then the paw edema is assessed as described in formaldehyde induced model at specific time points. Later on the last day of the experiment, the animals are sacrificed and hind paws are isolated for estimation of inflammatory biomarkers like tumour necrosis factor-alpha (TNF-α) and prostaglandin E2 (PGE2) in the paw inflammatory exudates by enzyme linked immunosorbent assay (ELISA) kit.[127]

*C) Complete Freund's Adjuvant (CFA)-induced arthritis model*

This model is commonly known as Adjuvant arthritis. It is the first model of RA and came into picture since Jules Freund in 1947 developed a mixture of heat-killed mycobacteria, mineral oils, and emulsifying agent. The mixture was termed as complete Freund's adjuvant (CFA).[128] This mixture when injected into the animals, preferably mice and rats, causes a systemic acute inflammation via the immune response mediated by T-cells. The inflammation progresses to polyarthritis that mimics the symptoms of human RA like joint swelling, lymphocyte infiltration and cartilage degradation.[129] In some cases, the repeated immunization leads the mycobacteria to cause persistent infection leading to mycobacterium and this mixture is called as incomplete Freund's adjuvant.[130] In this model, the arthritis is induced by intradermal injection of 0.05 to 0.1 ml of CFA at the sub plantar section of the right foot generally at 24 h after administration of the test substance. The paw edema volume is measured using a digital Vernier calliper or Plethysmometer at designated time interval during the study course of 14 days. Then at the last day, the animals are put through assessment of various parameters such as haematological parameters, especially haemoglobin (Hb), RBC, WBC, erythrocyte sedimentation rate (ESR) and platelet count. Then the animals are generally sacrificed for other evaluation like organ weight mainly thymus and spleen, because they play an important role in immunological process. Also, the ankle joint is isolated for histological analysis to check the presence of hyperplasia of synovium, inflammatory cells and fibrosis.[24,125]

## *Experimental animal models dealing with disease pathology-related aspect of RA*

The disease pathology related experimental animal models of RA resemble the histopathological features of human RA. In this section, some commonly used *in vivo* experimental models are discussed briefly.

*A) Antigen-induced arthritis*

Antigen-induced arthritis (AIA) models have been used widely for many years on mice and rats as it resembles histopathological features of human RA. In this model, the inducer agent is an exogenous antigen like serum albumin (mBSA) or ovalbumin mixed with CFA. The inducer agent is inoculated at the base of the animal's tail, followed by re-induction with the same antigen at the knee joint after 3 weeks to induce local inflammation.[124] The underlying principle is that the antigen like mBSA is cationic in nature. Hence, they bind with the negatively charged cartilage and get retained in the joint. As a result, the Arthus reaction is developed on articular cartilage due to development of antibodies against the retained antigens.[128] Therefore, this model helps in studying and understanding the hyper-reactivity of local T-cells to retained exogenous antigens.[131]

*B) Collagen-induced arthritis*

Collagen-induced arthritis (CIA) is the most predominantly used models of RA. In this model, the disease is induced by intradermal injection of an emulsion of type II collagen (CII) in CFA.[132] In response to this autologous collagen, the adaptive immunity produces anti-CII antibodies, which lead to polyarthritis in articular and periarticular structures within 21-25 days of first immunization. The mainly affected parts are front and hind paws. At extreme conditions, spinal columns and tails are also affected.[123] The animals are observed for two weeks after immunization during which period the paw edema volume are observed and recorded. In this model, the severity of joint destruction are also assessed using 3D micro-CT and the evaluating parameters are bone volume (BV), bone surface area/ BV (BS/BV) and trabecular thickness (Tb.Th). On the last day, the animals are sacrificed to isolate their knee joints for histological and immunohistochemical examination.[133] The source of collagen includes chick, porcine, bovine, human. Hence, the response depends on the strain and injection conditions.[132] CIA mimics many pathological and immunological features of human RA, for examples, breakage of self-tolerance, targeted cartilage immunity, B-cell activity and T-cell activity. Thus, it is an ideal model to study the pathogenesis for developing therapeutical intervention of RA.[124,134] Although this model was first described in Wistar and Sprague-Dawley rats, there are some genetically susceptible strain of mice such as B10.Q, DBA/1, and B10.RIII.[132] However, a drawback of this model is the issue of homogeneity in the pathogenesis and course of disease that arises due to the variation in the origin of CII, genetic background of the strain and environmental factors.[134,135]

*C) Spontaneous arthritis models*

In some genetically modified mice, arthritis occurs spontaneously as these mice are either transgenic or deficient of a specific gene of interest. These models are expected to provide meaningful information about genes associated in the inflammatory process. Thus, these models provide a tool to investigate the efficiency of therapeutics on the spontaneous development of joint inflammation in mice. The two most popular models are K/BxN mouse model and human tumour necrosis factor-transgenic (TNF-Tg) mouse model.[124]

*C1. The K/BxN model*

The K/BxN is a transgenic mouse found accidentally by the group of Kouskoff as a result of crossing between KRN mice with non-obese diabetic mice (I-$A^{g7}$). The resulting mice strain (K/BxN) developed arthritis spontaneously at the age of 4-5 weeks and the induction of arthritis is believed to cause by an immune response to the enzyme glucose-6-phosphate isomerase (G6PI).[136,137] In this model, severe symptoms of arthritis progressively evolve to a severe chronic inflammatory arthritis, which is similar to human RA in many important aspects as in the pannus formation, articular erosions, and inflammatory infiltration. Therefore, K/BxN mice mimic human RA in both aspects of autoimmune pathophysiology and key disease features.[138]

Also, the serum from K/BxN mice are extracted and transferred to other mice strains like BALB/c, CBA and C57BL/6 so that the effector phases study is not limited to particular genotype. The extracted serum from the K/BxN mice is injected twice (on day 0 and day 2 or 3), although some studies use single injection. The rapid swelling appears after 1-4 days, peaks after 10–14 days and resolves slowly within 2–3 weeks as the antibody clears off unless more serum is supplied.[139]

*C2. Tumour necrosis factor-transgenic (TNF-Tg) mouse model*

TNF-Tg mouse is a genetically modified mouse which was developed in the early 1990s. It over-express the human TNF-α transgene. 3647 and 197 strains of TNF-Tg are two commonly used transgene, where 3647 strains display milder arthritis as it contains one copy of the transgene. Whereas, the 197 strains display severe arthritis due to the presence of multiple copies of transgene.[140] This transgenic mouse exhibits symmetrical polyarthritis along with the proliferation of synovium as well as the infiltration of lymphocytes and polymorphonuclear cells in synovium space in every stage from the onset. In the later stage of the disease, severe arthritis symptoms like formation of pannus, destruction of articular cartilage and fibrous tissue are developed.[141] The model has been found to be useful in determining the varying attributes of effector cytokines in regulating inflammation as well as in cartilage and bone destruction. Hence, TNF-Tg mouse is an ideal model to investigate TNF-induced inflammatory pathways in human disease.[124] Table 4 briefly represent the applications of these *in vivo* RA models in the development of anti-arthritic therapies reported in literature.

As the modelling of arthritis dates back to many centuries, the availability of various models is abundant and a pattern is observed where the shortcomings of particular model are compensated by the new and more developed models. Few arthritic models like adjuvant arthritis, collagen-induced arthritis, streptococcal cell wall arthritis and antigen-induced arthritis are the most historically prevalent models of arthritis as they are the main choice of arthritis models in animals according to the reported literatures in the past few decades. However, they are still relatable with current arthritis research.[142,143] It is observed from the literature that big animal models for arthritis are less reported currently owing to the ethical issues, longer aging and cost effectiveness. Such big animal

**Table 4.** Efficiency of *in vivo* RA animal models in paving the way of anti-arthritic interventions to clinical use.

| Type of *in vivo* RA model | Therapeutic intervention | Description | Ref. |
|---|---|---|---|
| Collagen-induced arthritis in mice | Prednisolone | Inhibited paw inflammation in developing disease | 144 |
| Collagen-induced arthritis in mice | Cyclosporine A | Decreased severe arthritis | 145 |
| Collagen-induced arthritis in mice | Cyclophosphamide | Decreased severe arthritis | 145 |
| Adjuvant-induced arthritis in DA rats | Gold sodium thiomalate | Reduced the severity of arthritis when given a week before adjuvant | 146 |
| Adjuvant-induced arthritis in rats | IL-17 receptor/human IgG1 Fc fusion protein (muIL-17R:Fc) | Attenuated paw volume in dose dependent manner | 147 |
| Collagen-induced arthritis in rats | Methotrexate | Reduced arthritic score and paw diameter | 148 |
| Collagen-induced arthritis in mice | Etodolac | Prevented the development of synovitis, erosion of cartilage of the joints and bone destruction | 149 |
| TNF-Tg mouse model | Diacerein | Inhibited the onset of arthritis and attenuated the progression of arthritis | 150 |
| TNF-Tg mouse model | Dexamethasone | Decreased disease progression | 150 |
| Collagen-induced arthritis in mice | Human mesenchymal stem cells | Reduced joint inflammation and damage | 151 |
| Collagen-Induced arthritis in rats | Lyophilized curcumin extracts with iontophoresis | Ameliorated arthritic symptoms significantly | 152 |
| Carrageenan- induced rat paw edema model and CFA-induced in rats | *Terminalia tomentosa* bark | Reduced paw edema volume in both models | 153 |

models include dog RA models, monkey RA models, goat arthritis models, pig arthritis models and horse OA models.[36,129] Among chemically induced arthritis models, papain induced arthritis model has historical use. However, it is less frequently used currently.[102]

Hence, it is clear that the experimental arthritis animal models act as a cornerstone in the field of biomedical research to study the concepts and their association with each other; to encourage the enthusiastic mind to find other ways that can dissect the disease into simpler mechanisms; to identify targets; and finally develop the right and most effective therapeutic intervention to circumvent this life-threatening disease.

## Ethical Concerns and Possible Refinement of Animal Usage

The contribution of animal models in finding various biomedical research questions over the time is unquestionable and enormous despite having some skepticism over the extrapolation of clinical relevancies based on the data of animal models. However, it has increased the concern of the welfare of animals involved in the experimental research. This issue has focused the attention of animal rights activists as well as animal lovers to the related ethical issues concerning these experimental protocols involving animals.[153] Over the years, many concerned authorities and organisations have laid down certain protocols and measures to be followed by the researchers while performing *in vivo* experimental work involving animal models. There are many such regulations which fall under the legislation of a particular geographical region or for the entire globe; it is the sole responsibility of the researcher to select the best suitable and compatible guidelines to incorporate in his/her work without compromising the scientific soundness of the work. Hence, adequate technical knowledge of the experiment paired with safety concerns of the animals will produce fruitful and ethically sound research work.[154]

Dating back to the history, the upsurging sentiments of the antivivisectionists of Victorian Britain had led the British Parliament to introduce The Cruelty to Animals Act in 1876. The Act includes the regulations on animal experimentation.[155] The three main highlights of this Act were: (a) animal experiments should only be carried out when there is absolute need of knowledge that will be useful for saving or prolonging life or alleviate suffering; (b) the animals must be anesthetized; and (c) the animals must be killed immediately after the experimental procedure if they would be injured or in pain as a result of the experiment.[152] Another one is the Principle of 3R (Reduction, Replacement and Refinement) introduced by Russell and Burch in 1959, where each R represents universal principles for ethical use of animals in experimental work and is being followed by many countries.[156]

In 2010, the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines were published as an initiative of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). These guidelines have been translated into several languages and entail a checklist consisting twenty key information in order to produce high-quality research that can be reviewed and reproduced. Information can be the number, species, strain, sex, genetic traits of the animal to be used for study including the husbandry and housing; and the experimental, statistical, and analytical methods.[158]

The use of animals for *in vivo* study of arthritis plays an indispensable role in the development of anti-arthritis interventions. The methods and experimental protocols to be involved in animal study must be transparent, translational and must be based on a "harm-welfare assessment". The potential harm involves in the ways used to induce arthritis in animals that can cause pain and suffering, which may be mild, moderate or severe based on the model and the duration of the study.[158] It is also definitely necessary to consider the mechanistic translatability and applicability of the various in silico, *in vitro* and clinical methodologies used to address scientific questions associated with arthritis. Wherever feasible, replacing or avoiding animal use should be the prime goal as demanded in many legislations including European Directive 2010/63/EU (European Commission 2010).[159] Hence, it is imperative to adopt such techniques that will enable to avoid, reduce, replace or avoid the involvement of animals like skimming out the compounds that have no potential therapeutic benefit. And if there is no such viable alternative approach, the selection of animal models to be used must be approved by the animal welfare authorities. It will not only minimise the suffering but also fulfils the scientific purpose.[160] The exasperated and complicated process of arthritis-based research requires large number of animals which goes through suffering due to the disease induction process. This calls for the refinement of these animal models in arthritis-based research. Table 5 enumerates a few ways that can be adopted in order to refine the number of animals.[159]

## Current Status Quo of Clinical Trial Updates on Arthritis

A draft containing the clinical investigation of RA medications other than non-steroidal anti-inflammatory drugs (NSAIDs) has been opened by the European Medicines Agency (EMA) for public consultation. Some of the undergoing clinical trials on new arthritic therapeutic interventions are listed in Table 6.[161]

## Conclusion

Understanding the underlying molecular and pathophysiological mechanisms of any diseased condition is the foremost step in the development of a new therapeutic intervention. So far, many *in vitro* and *in vivo* arthritis experimental models have been developed each with their own significances and shortcomings. It is observed that larger animal models mimicking arthritis are no longer preferred along with certain chemically

**Table 5.** Refinement of animal models in arthritis-based research.

| Ways of refinement | Details |
|---|---|
| Housing and care refinements | Appropriate and suitable environment (soft litter to avoid pain while walking, soft, non-tanGling nesting material, etc.)<br>Ambient temperature (30-31℃)<br>Adequate food and water<br>Distraction from pain |
| Way of catching animals | Avoid catching by the tail of rodents<br>Use cupped hands while catching |
| Arthritis inducers and their administration | Reducing the dose, volume and frequency<br>Nature of antigen, adjuvant and solvent<br>Avoid "tracking" (tunnel remains under the skin after withdrawal) by wide gauge needles while administration<br>Avoid intradermal injection closer to tail which might cause ulcer |
| Study duration | Reduce the duration of the study as long as it is compatible with the study objective and statistically significant data can be obtained during that period of time |

**Table 6.** Anti-arthritic therapeutic interventions under different phases of clinical trials.[161]

| Study title | Intervention | Arthritis type | Phase | Sponsor | CT identifier |
|---|---|---|---|---|---|
| A Phase 2 Study of Hemay007 in Patients With Rheumatoid Arthritis | Hemay007 800 mg | Rheumatoid arthritis | Phase 2 | Tianjin Hemay Pharmaceutical Co., Ltd | NCT05247216 |
| Efficacy and Safety of Baricitinib for the Treatment of Moderate to Severe Rheumatoid Arthritis | Baricitinib | Rheumatoid arthritis | Phase 4 | Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh | NCT05660655 |
| Tofacitinib Versus Methotrexate as the First Line DMARD in the Treatment of Rheumatoid Arthritis | Methotrexate<br>Tofacitinib | Rheumatoid arthritis | Phase 4 | Dr. Mohammad Mamun Khan | NCT04464642 |
| Tetrandrine in the Treatment of Rheumatoid Arthritis | Tetrandrine | Rheumatoid arthritis | NA | Peking University People's Hospital | NCT05245448 |
| Efficacy and Safety of RTX-GRT7039 in Adult Subjects With Knee Osteoarthritis | RTX-GRT7039 | Osteoarthritis | Phase 3 | Grünenthal GmbH | NCT05248386 |
| Platelet Rich Plasma (PRP) Injections For Knee Osteoarthritis | PRP injections | Osteoarthritis | Completed | Azienda Ospedaliero-Universitaria Consorziale Policlinico di Bari | NCT04852380 |
| Evaluation of Safety and Exploratory Efficacy of CARTISTEM®, a Cell Therapy Product for Articular Cartilage Defects | CARTISTEM® | Osteoarthritis | Phase 1<br>Phase 2 | Medipost Co Ltd. | NCT01733186 |
| Role of L-Arginine Supplementation in the Treatment of Rheumatoid Arthritis | L-arginine | Rheumatoid arthritis | Phase 2 | RenJi Hospital | NCT04535427 |
| Study Evaluating the Long-Term Safety and Efficacy of ABX464 in Patients With Moderate to Severe Rheumatoid Arthritis | ABX464 | Rheumatoid arthritis | Phase 2 | Abivax S.A. | NCT04049448 |
| Safety, Tolerability, Pharmacokinetics and Pharmacodynamics Study of VAY736 in Rheumatoid Arthritis Patients | VAY736 | Rheumatoid arthritis | Phase 1 | Novartis Pharmaceuticals | NCT02675803 |

induced models like papain induced arthritis. Adjuvant arthritis, collagen-induced arthritis, streptococcal cell wall arthritis, and antigen-induced arthritis are mostly prevalent models of arthritis. But owing to the complicated disease etiology of arthritis, the availability of the perfect simulation models mimicking the healthy and diseased joint physiology is very few. This limitation of the existing animal models has therefore led to look for more sophisticated *in vitro* models that can more accurately mimic the *in vivo*-like environment. The progress in the domain of bioengineering has facilitated the development of *in vitro* models that reiterate the complex 3D organ-level structures with integrated biochemical, physical and mechanical stimulation. Ultimately, the development of an advanced *in vitro* model of the joint will assist in reducing or replacing the use of animal models in biomedical

research, personalized medicine and pharmaceutical formulation development. To date, there is no single ideal experimental model that covers all features of both RA and OA, and consideration of the advantages and disadvantages of each model is instrumental when designing a study. In order to ensure that the animal involving experiments are controlled, well-designed, and well-evaluated, it is important to consider the ethical issues like the importance of 3Rs. A brief update on clinical trials of anti-arthritic therapeutics has also been included. Therefore, there is a need for aggressive foraging for ideal arthritis models those are translatable to human condition.

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## Author Contributions

L Ronibala Singha: Investigation, Formal analysis, Writing - Original Draft. Malay K Das: Conceptualization, Supervision, Writing - Review & Editing

## Conflict of Interest

The authors report no conflicts of interest.

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